Chapter 15

VETERINARY PATHOLOGY

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INTRODUCTION

"*Taceant colloquia. Effugiat risus. Hic locus est ubi mors gaudet succurrere vitae.*"
"Let idle talk be silenced. Let laughter flee. This place is where Death delights in succoring life.")

—Anonymous Latin quotation often attributed to Giovanni Battista Morgagni[1(p126)]

During the 18th century (the Age of Enlightenment), medicine and the study of disease underwent extraordinary changes. Previously entrenched beliefs in imbalances and inconsistencies in the body's "humors" as the cause of disease shifted to a more scientific, rational system of pathogenesis as the true source of health-related maladies. Giovanni Battista Morgagni, considered by some to be the father of anatomical pathology,[2] was among the pioneers of this change in thinking, and it was through his study of disease that the foundation of the veterinarian's work, with the delicate interplay of diagnoses, prognoses, and treatment of illness, became irrevocably interwoven with a thorough and consummate understanding of the origin, nature, and course of disease. His early work also helped pave the way for others such as John Hunter, Claude Bourgelat, and Rudolph Virchow, to expand and ingrain the critical study of disease pathogenesis into the medical community.[3]

Hunter is known as the founder of pathological anatomy in England; Virchow as being the German physician who advanced public health; and Bourgelat as the French veterinary surgeon who advocated veterinary colleges in Lyon, France.

Because of Bourgelat's persistence, the first internationally recognized school of veterinary medicine was established by King Louis XV in Lyon, France, in 1761.[4] The school emerged as an extension of the Lyon Academy of Horsemanship, with the inherent mission of producing an educated group of individuals capable of giving proper medical care to the horses of both the military and the gentry. Implicit in this mission was eradicating the rinderpest virus, a highly virulent morbillivirus known as the cattle plague or *steppe murrain*. Centuries later, in 2011, this virus became only the second disease in human history to be eradicated.[5]

During the 18th and early 19th centuries, American veterinary students studied in Europe or underwent coursework in institutions on the East Coast; almost 100 years passed from the founding of the school in Lyon to the establishment of the first American veterinary school. In 1852 the Veterinary College of Philadelphia was created, followed by the Boston Veterinary Institute (1854) and the New York College of Veterinary Surgeons (1857).[6,7] Although veterinarians were well-established within the European militaries, the US Army did not establish regulation specific to veterinary medicine until 1835 and only began appointing veterinary surgeon graduates from accredited institutions in 1879.[7] Despite the late start of the US Army Veterinary Corps (VC) in comparison with its European counterparts, the VC's contributions to military medicine, in general, and to pathology, in particular, have made up for lost time. (For more information about the VC's contributions to military medicine throughout history, see also Chapter 1, Military Veterinary Support Before and After 1916.)

The current US Army military veterinary pathologists (area of concentration 64 D or "64 Delta") are a diverse group of officers who contribute to the overall health and well-being of US service members. Their support of and integration into military medical research remains critical to the continued production of medical countermeasures such as vaccines, therapeutics, and medical devices to combat various disease and nonbattle injuries. Military veterinary pathologists are also in the first line of defense in diagnosing both veterinary-specific and zoonotic (ie, affecting both human and animal) diseases that could represent a threat not only to deployed forces, but also to the US population.

In 1980, the US Army Veterinary Services began providing all veterinary-related functions for every aspect of veterinary support for all military branches. The military veterinary pathology specialty has become refined over time and across continents into a small but extremely effective force multiplier within the VC. The "64 Deltas" train their own members through a residency program; support medical research in the development of chemical, biological, radiological, and nuclear (CBRN) medical countermeasures; sustain the military working animal mission through diagnostic assays; and deploy with the service members of all DoD services in various diagnostic support roles. This chapter will discuss the diverse roles of the veterinary pathologist within the US military of the past, present, and future.

DIAGNOSTICS

Definitions and Scope of Diagnostics in Veterinary Pathology

Pathology is the study of disease. As a medical specialty, pathology can be conceptually classified under various rubrics; for instance, pathology may be classified based on an organ system (eg, ocular, pulmonary, or neuropathology); or on groups of etiologies (eg, toxicologic or environmental pathology); or even by the species studied: medical pathologists study human disease, whereas veterinary pathologists generally study nonhuman, animal disease. But because they study diseases that affect all animal species, including humans, most military veterinary pathologists are traditionally considered comparative pathologists.

Functionally, pathology can be classified based on the nature of work supported. Examples include experimental, clinical, general, and diagnostic pathology. Diagnostic pathology is the study of tissue abnormalities at the gross (ie, macroscopic), histologic (ie, microscopic), ultrastructural, and molecular levels, in order to identify the nature of disease, and thereby make a diagnosis.[8] What follows is a brief history of diagnostic veterinary pathology in the US military, punctuated by selected achievements and contributions to the science as a whole; subsequent sections of this chapter address other functional areas of military veterinary pathology. The two overarching themes of this section are (1) that diagnostic pathology forms a cornerstone for both the education and practice of military veterinary pathology and (2) that military accomplishments in diagnostic pathology have not only contributed to the greater body of biomedical knowledge, but have also shaped the specialty of veterinary pathology as it is now organized in North America.

Diagnostic pathology was the primary professional forte of the specialty's early pioneers; their efforts were driven by an urgent need to understand the pathogenesis and, thereby, the treatment and prevention of diseases affecting the livestock that society and the military depended on. The US military led the early evolution of veterinary pathology in the United States; the development of US veterinary pathology is, therefore, inextricably linked to that of military veterinary pathology.

Expertise in diagnostics is also an indispensable attribute of today's US Army veterinary pathologists, regardless of duty assignment. Army veterinary pathologists provide diagnostic pathology support for all military working animals, including dogs in the DoD and other federal agencies; caisson and ceremonial horses; and marine mammals such as dolphins and California sea lions used by the Navy for search and recovery missions. Additionally, through the DoD Veterinary Pathology Residency (DODVPR), the postgraduate training program through which nearly all Army veterinary pathologists since 1983 have been trained in their specialty, Army veterinary pathologists provide diagnostic support for research animals within the DoD and other federal agencies, pets owned by eligible military service members, and second-opinion cases referred by civilian and military veterinary pathologists worldwide.

Moreover, the American College of Veterinary Pathologists' (ACVP) board-certifying examination emphasizes diagnostic expertise. Since certification is among the major goals of residents in the DODVPR and a requirement for continued specialization in the area of concentration 64D, diagnostic proficiency ultimately forms a foundation for the practice of military veterinary medicine and for more specialized work in the comparative and experimental research arenas.

Historical Background of Diagnostics and Early Contributions to Veterinary Pathology

US military work in veterinary pathology began with a spotlight on diagnostics at the Army Medical Museum (AMM), a center established during the Civil War specifically for the collection of specimens for research in military medicine and surgery. Officially, veterinary activities at the AMM (redesignated the Army Institute of Pathology AIP in 1946 and redesignated again in 1949 as the Armed Forces Institute of Pathology AFIP to recognize its status as a triservice organization[9]) did not begin until 1943, when Major Charles Louis Davis became the first veterinary pathologist assigned to the AMM.[10] However, research in veterinary pathology at the AMM actually began much earlier: almost immediately after its organization by the Army surgeon general, Brigadier General William Hammond, in May 1862. By 1867, just 5 years after its founding, the AMM inventory already included specimens from a variety of animal species.[2] By 1876, the AMM's comparative anatomical section listed 1,522 specimens.[9]

The first person in the United States to conduct veterinary pathology research was the physician Joseph J. Woodward. Woodward volunteered for military service at the onset of the Civil War, was commissioned in the US Army Medical Department (AMEDD) in 1861, and was assigned by Surgeon General Hammond as the first medical pathologist

at the new AMM in 1862.[11] During the 1860s, a devastating epizootic of contagious pleuropneumonia of cattle prompted the US Commissioner of Agriculture to engage visiting professor John Gamgee, of Edinburgh, as a consultant to study the disease. Gamgee submitted tissues from affected animals to Woodward at the AMM, where Woodward performed the first histological study of the disease and submitted his report, complete with photomicrographs, to the US Commissioner of Agriculture in 1870. Woodward's report on bovine pleuropneumonia is considered to be the first scientific contribution to veterinary pathology in the United States.[11]

Woodward later published a comparative report on the erythrocytes of humans and various mammals, particularly noting similarities with those of the dog. He went on to achieve recognition as a photomicrographer, bibliographer, and pathologist. In fact, he is the only pathologist to have participated in the autopsy of two American presidents (Abraham Lincoln and John Garfield), and his acclaimed *Medical and Surgical History of the War of the Rebellion*, praised by Rudolph Virchow, "the father of modern pathology," is considered by some to be the most important contribution ever made to military medicine.[11]

Figure 15-1: Dr Thomas Carlyle (TC) Jones.
Photograph courtesy of the National Museum of Health and Medicine archives.

In the years following Woodward's report on bovine pleuropneumonia, medical pathologists became more curious about the veterinary pathologists assigned to the AMM. However, it was not until 1943 that veterinary pathology became an official capability of the AMM. Colonel James Earle Ash, Medical Corps, US Army, served two terms as curator (the title was changed to director with the AMM's name change to the AIP in 1946) of the AMM, from 1929 to 1931 and 1936 to 1946.[12] During the second of these terms, Ash mentored and supported a young Thomas Carlyle (TC) Jones (Figure 15-1), who was stationed as a veterinary officer at the Army Veterinary Research Laboratory in Front Royal, Virginia, conducting research on equine diseases of importance to the military at war.

Jones was particularly interested in equine ocular pathology and visited the AMM frequently to consult with the medical pathologists assigned there. Ash, a medical doctor who attributed his own favorable disposition toward veterinary medicine partly to his marriage to the daughter of a prominent veterinary professor at Cornell University,[13] collaborated with Jones, and the two coauthored a seminal paper on the histopathology of equine periodic ophthalmia (ie, equine recurrent uveitis).[12] Jones later wrote an editorial to honor Colonel Ash on his 100th birthday,[13] in which Jones emphasized the unique collaborative culture at the AMM. At that time, medical and veterinary pathologists were segregated as a matter of course; yet, at the AMM, Jones found a welcoming group of devoted professionals eager to share their expertise with the young veterinarian.

By the early 1940s, the AMM already housed several special pathology registries (ie, collections of histologic slides defining diseases with associated case descriptions) supported by various medical specialty societies. Jones proposed to Ash the establishment of a registry for veterinary pathology, an idea Ash had already envisioned. In 1943, with the support of the preeminent veterinary pathologist in the country at that time, William H. Feldman of the Mayo Foundation, Ash gained the backing of the American Veterinary Medical Association and the Army surgeon general to found the Registry of Veterinary Pathology at the AMM. In doing so, Ash officially made a place for veterinary pathology in the world's largest, most productive pathology institute, setting the stage for Jones' formal establishment of the specialty of veterinary pathology in North America.[13]

At the Registry's helm during these groundbreaking events was the previously mentioned Major Charles Louis Davis, an Army reservist and pathologist with

the US Bureau of Animal Industry, who had been called onto active duty during World War II and served as the first registrar from 1943 to 1945. At the close of the war, Davis was demobilized and succeeded as registrar by Major TC Jones.[12] The written transcript of Jones' interview by Charles S. Kennedy (AFIP Oral History Program, Atlanta, Georgia, March 17, 1992) reveals that by the time Jones reported to the AIP in 1946, there were already 10,000 to 20,000 cases in the fledgling registry, thanks to Davis' efforts.[14] Davis was later elected a charter member and fifth president of the ACVP.[10] Today, the nonprofit Charles Louis Davis DVM Foundation for the Advancement of Veterinary and Comparative Pathology supports veterinary and comparative pathology internationally through a variety of educational outreach programs.

Taking over after Davis, Jones continued to nurture the Registry of Veterinary Pathology as it grew into a robust collection. He personally contributed equine specimens from the research laboratory in Front Royal, where he was previously assigned, and began to add material from war dog centers, such as those at Front Royal and Ft Robinson near Crawford, Nebraska. He further expanded the Registry by offering diagnostic consultation to civilian veterinarians and veterinary pathologists in exchange for submitted specimens, beginning a tradition of symbiosis with the veterinary community at large that became a mainstay of the program for generations. In his AFIP interview, Jones recounted the fervor among medical pathologists for comparative pathology during the mid-1940s, around the time he arrived as the Registry of Veterinary Pathology's second registrar. He quipped, "It wasn't my charm or anything of the kind. The reason that my colleagues, the [medical] pathologists, were interested in the material that I showed them was that it gave them fresh ideas. . . ."[14(p7)]

The collaboration between veterinary and medical pathologists at the AIP soon became evident in the scientific literature, where medical advances that resulted directly from this new cooperation were published. For example, in those days, pathologists from medical schools throughout the United States came to the AIP as consultants. One such pathologist, Henry Pinkerton, reported on the similarities between the microscopic lesions of human measles and canine distemper.[15] From this early observation, scientists later discovered that the diseases are caused by two closely related morbilliviruses.

Another important contribution made in comparative pathology at the AIP during this period was the discovery by ocular pathologist Helenor Campbell Wilder Forester of ocular larval migration of *Toxocara canis* nematodes in the eyes of human infants. At a staff conference, Wilder presented granulomas in the eyes of infants who had been enucleated in response to a clinical suspicion of having retinoblastoma, a form of ocular cancer. Jones noted the lesions' striking resemblance to canine ocular granulomas caused by nematode larval migration,[14] prompting Wilder to reevaluate her specimens, ultimately leading to her discovery of the larval form of *Toxocara canis* in these eyes. Consequently, effective therapeutic and public health preventive measures were employed to combat the blinding disease.[16] In his biography of James Earle Ash, Leon Saunders summarized the unique collaborative culture of the AIP in the early years after veterinary pathology was formally added to the Institute: "In no other institution in the world did veterinary pathologists have the opportunity to work in such close contact with so many human pathologists. . . .[I]n no other institution were the daily activities (of diagnosis, research and teaching) such a patent validation of Virchow's concept that '*there is only one medicine*.'"[12(p446)]

Although Ash, Davis, and Jones all helped shape the Registry of Veterinary Pathology, TC Jones is credited with the establishment of veterinary pathology as an organized specialty in North America.[12] Born in 1912 in Boise, Idaho, Jones was heavily influenced in his interest in animals by years spent living and working on a nearby dairy farm owned by family friends. He received his DVM degree at Washington State College in 1935. During veterinary school, he worked under Hilton A. Smith in the pathology department, where Jones developed an interest in pathology and an awareness of the VC. Because he was without direct connection to the Army, Jones wrote a letter expressing his interest in joining the VC to (then) Lieutenant Colonel Raymond Kelser, who had authored a veterinary textbook that Jones used and liked. Buoyed by Kelser's response, Jones sat for the highly competitive VC examination at Ft Douglas, Salt Lake City, Utah, and accepted a commission on October 1, 1935. (Kelser was eventually promoted to the rank of brigadier general and served as the VC chief from 1938 to 1946.)[14]

Recognition of veterinary pathology as an organized specialty in North America began with Jones' observation of young medical officers fervently studying at the AIP for their board-certifying examinations. Admiring their diligence, Jones envisioned the potential such a stimulus might provide towards advancing the field of veterinary pathology. Again supported by fellow pioneer William H Feldman, who by now was a consultant to the AIP, Jones gained the American Veterinary Medical Association's approval for the establishment of its first specialty group, the American College of Veterinary Pathologists (ACVP). The ACVP was born in a Chicago hotel room, where

15 pathologists, including Jones and Davis, met in 1948. Among the ACVP's 42 charter members were Jones (secretary-treasurer), Davis, Feldman (president), and Smith.[17] From these humble beginnings grew today's ACVP, which now encompasses some 1,400 active members and 140 emeritus members.[18]

Jones remained at the AFIP until 1950, when he was reassigned to West Germany; while stationed there, he became the first American elected to West Germany's veterinary pathology association and promoted the veterinary pathology activities of the AFIP, even publishing a German article on the topic.[12] He returned as chair of the Veterinary Division at the AFIP from 1953 to 1957, during which time he established the AFIP's first course on laboratory animal diseases, which was used for many years as an educational program for pathologists and laboratory animal veterinarians. The course remains a staple in veterinary pathology training and is now run by the Charles Louis Davis DVM Foundation for the Advancement of Veterinary and Comparative Pathology. Also, during his chairmanship, Jones co-authored the classic text *Veterinary Necropsy Procedures*, published in 1954, and began writing what would become the definitive textbook for veterinary pathology education, *Veterinary Pathology*, coauthored with Smith and first published in 1957.[12]

Lieutenant Colonel Jones retired from the Army in 1957, launching a second career that included distinguished service at Angell Memorial Hospital in New York, New York, and Harvard University in Cambridge, Massachusetts. Among his other lasting contributions to the specialty were co-writing authoritative texts on laboratory animal pathology, writing six editions of his *Veterinary Pathology* text (the last of which was published in 1996) and serving a term as president of the US and Canadian Division of the International Academy of Pathology.[12,19]

Evolution of the Department of Defense Veterinary Pathology Residency Program

Jones also merits recognition for initiating what would evolve into a rigorous postgraduate training program in veterinary pathology (DODVPR). During his first assignment at the AIP, Jones started a training program in which veterinarians could both learn pathology and obtain a graduate degree from George Washington University; the first graduate of the program, Andrew W. Monlux, earned his doctorate in comparative pathology.[12]

In the *Report of the Committee on Registry of Veterinary Pathology for 1946*, an internal account written annually for the AIP (and later, the AFIP), committee members recognized both the tremendous educational value of the Registry that Jones nurtured and the unique opportunity afforded by the AIP's learning environment and advanced specialized training: " . . .[T]o best serve the veterinary profession, the Registry should be in a position to supply deficiencies in the teaching collections of the respective veterinary colleges and to provide facilities for graduate training in pathology. . . .The [AIP] offers opportunities for this training to a degree found nowhere else. It is exceedingly important that the Registry of Veterinary Pathology have at all times a graduate student assigned for a year's training in this unusually fine institution."[20(pp1–2)] Although the original program lapsed during Jones' tour in West Germany, Jones revived the residency for veterinary officers in the Army and Air Force when he returned in 1953 as chair of the Veterinary Division.[12]

By 1960's *Annual Report*, the training program, hosting seven duty and resident officers working toward qualification for certification by the ACVP, was a 2-year residency consisting of "supervised casework, daily slide conferences, a minimum of three seminars a week, gross necropsies, numerous short courses available at the AFIP," and integration into ongoing research projects at the Institute.[21(p3)] As the demand for veterinary pathologists in military medical research and diagnostic medicine increased, a preceptorship was developed in 1967 to train veterinary pathologists at various military research sites, of which the AFIP was just one. By the 1980s, however, leaders in military veterinary pathology advocated for the consolidation of all veterinary pathology training into a single 3-year residency program; as a result, The Army Surgeon General Lieutenant General Berhnard Mittemeyer established the DODVPR at the AFIP in October 1983. From then on, nearly all military veterinary pathologists were trained through the 3-year DODVPR, which soon gained worldwide recognition as one of the largest and most effective programs of its kind.

The AFIP was closed in 2011 as the result of the Congressionally directed 2005 Base Realignment and Closure law. However, as a testament to its value as the main provider of ACVP board-eligible military veterinary pathologists to support and conduct biomedical research and diagnostics for the DoD, the DODVPR was preserved and aligned under the Joint Pathology Center (JPC). Established by the 2008 National Defense Authorization Act to serve as the federal government's pathology reference center providing diagnostic consultation, education, and research services to other federal agencies, the JPC assumed many of the core missions of its predecessor, the AFIP, including oversight of the DODVPR, which in June 2011 relocated from the AFIP in Washington, DC, to the JPC's Forest Glen Annex in Silver Spring, Maryland.

At the JPC, residents are afforded the opportunity to study in consultation with international medical subspecialty pathologists. Upon successful completion of the program, participants are eligible for board examination by the ACVP and are assigned as biomedical research pathologists supporting or conducting DoD biomedical research as diagnostic pathologists or as staff members responsible for training new residents in the DODVPR.

New residents are competitively selected VC officers who apply through the Army's Long-term Health Education and Training program. Usually four new residents are assigned each year, for a total of 12 residents in training at any given time. Before entering the program, residents have typically completed two assignments, including at least one overseas or operational tour and approximately 5 years of service in the VC.

The DODVPR's current structure resembles the earlier curriculum in many ways: at a minimum, the program consists of supervised casework; daily case "rounds" in which residents present and discuss diagnostic cases with staff pathologists; twice weekly seminars in systemic pathology; weekly seminars in gross, clinical, and general pathology; a weekly international histopathology slide conference (ie, the Wednesday slide conference or WSC discussed later in this chapter); textbook and journal reviews; an oral case presentation at the Northeast Veterinary Pathology Conference; a poster presentation at the ACVP conference; attendance at several pathology courses offered by the Charles Louis Davis DVM Foundation for the Advancement of Veterinary and Comparative Pathology; and participation in external rotations at the Smithsonian National Zoological Park, National Institutes of Health, Frederick Animal Health Diagnostic Laboratory, and University of Minnesota Veterinary Diagnostic Laboratory. Seven ACVP diplomates (ie, board-certified pathologists), two civilian and five military veterinary pathologists, lead this training.

Throughout its existence, the DODVPR has contributed to the veterinary pathology specialty both by training residents and by freely sharing its template for educational success with the broader veterinary pathology community, including the diverse array of specimens available in its Registry of Veterinary Pathology. These core training materials are reviewed methodically by incoming residents over the course of their residencies and are eventually organized by body system, a model which has proven highly effective in preparing candidates for the ACVP examination. Today, thanks to a 2002 US Department of Education grant, this material is also available on the Internet as *Veterinary Systemic Pathology Online*.[22]

The WSC also illustrates the symbiosis between the DODVPR and the broader veterinary pathology community. The conference dates to 1953; today, it consists of 25 conferences per year, each comprising four cases.

Cases for the WSC are contributed by veterinary pathologists in roughly 140 veterinary diagnostic and research institutes worldwide, representing academia, industry, zoological parks, and state and national governments and their agencies. Cases encompass a wide variety of classic, rare, and recently published diseases for which microscopic or ultrastructural diagnoses are achievable. Residents receive and evaluate the cases as unknowns and develop histological descriptions and diagnoses independently in preparation for the weekly conference.

Each conference is led by an invited moderator, including military and civilian veterinary pathologists, many of whom are experts in their fields; most moderators also provide additional educational seminars for residents and staff when visiting. Because residents are selected at random during the conference to present their findings, interpretations, and diagnoses, under the scrutiny of the moderator and staff, the conference provides a valuable tool for developing the diagnostic and interpretive skills and descriptive techniques vital to the practice of veterinary pathology and success on the ACVP-certifying examination.

Each year, the conference is coordinated by a second-year resident who collects, selects, and redistributes cases; invites and schedules moderators; and compiles and publishes the conference proceedings. Following traditions that began in the early years of recruiting cases to grow the Registry of Veterinary Pathology, the WSC embodies a close, cooperative, mutually beneficial relationship between the DODVPR and the veterinary pathology community at large. In exchange for submitting cases, participating institutions receive glass or digital slides for all 100 selected cases and the conference proceedings compiled at the conclusion of the training year; these, in turn, become training materials in the libraries of veterinary pathology training sites worldwide. Conference proceedings dating back to 1964 are freely accessible online, along with virtual slides for all conferences since 2007, essentially offering anyone in the world with Internet access the opportunity to replicate the experience of participating in and learning from the WSC.[23]

Several more examples illustrate the strong collaboration between the DODVPR and the veterinary pathology community. In exchange for the opportunity for DODVPR residents to perform necropsies on a variety of species at the Smithsonian National Zoological Park, the civilian residents studying at the zoo are invited to participate in all training curriculum

at the DODVPR. Similarly, beginning in 2009, the DODVPR began sending second-year residents for a 2-week rotation at the University of Minnesota Veterinary Diagnostic Laboratory to conduct necropsies in an academic setting; in return, residents from the University of Minnesota visit the DODVPR near the completion of their training program in preparation for the ACVP-certifying examination. Also, veterinary pathology residents and veterinary students from around the world visit the DODVPR to personally use the extensive training materials available for study.

Selected Military Achievements in Diagnostic Veterinary Pathology

The selected achievements listed in this section of the chapter provide only a few examples from military veterinary pathology accomplishments, particularly as related to the care of military working animals. Military veterinary pathologists have provided diagnostic support to the military working dog (MWD) since the inception of the Registry of Veterinary Pathology, when TC Jones furnished diagnoses on cases submitted from war dog centers in exchange for submitted case material to the Registry.[14] By the early 1960s, the AFIP, recognizing the value in consolidating MWD case submissions in one place for study, recommended to the armed services that all surgical and necropsy material from military sentry and scout dogs be submitted directly to the AFIP; by 1966, such action was mandated.[24]

As the Registry of Veterinary Pathology became a viable source of case material representing diseases of MWDs, veterinary pathologists at the AFIP were able to diagnose and report on diseases of importance to the MWD population as a whole. For instance, military veterinary pathologists published papers on the pathology and pathogenesis of tropical canine pancytopenia (ie, canine ehrlichiosis) in the early 1970s after its diagnosis in MWDs deployed during the Vietnam War.[25–27] Most of the approximately 1,600 American MWDs that served in Vietnam were transferred to the Vietnamese or euthanized, the result of Army policies aimed at eliminating the threat of transmission of the disease, endemic in Vietnam, back to the United States.[28]

In 1978, during his third year of residency at the AFIP, another military veterinarian, Dr John Pletcher, diagnosed the first case of Chagas disease in an MWD in the United States.[29] While reviewing histopathology slides on an MWD necropsy case submitted from the dog center at Lackland Air Force Base in San Antonio, Texas, he spotted the tell-tale microscopic pseudocyst of *Trypanosoma cruzi* in the heart. Pletcher and collaborating clinicians assigned at the dog center determined that MWDs contracted the fatal disease by ingesting the vectors, triatomine bugs of the Reduviidae family, which dropped into their kennels after being zapped in the overhead lights; most affected dogs had oral mucosal lesions, a direct portal of entry for the protozoa to establish infection.

Pletcher also diagnosed an early case of canine parvoviral enteritis. At that time, canine parvovirus was just emerging in the United States, so when he observed intestinal lesions typical of feline panleukopenia (also caused by a parvovirus) in the intestines of submitted puppies, he first assumed that the consultation request form had been mislabeled and that he was actually looking at tissues from kittens. His eventual diagnosis confirming that the lesions came from puppy intestines soon led to one of the first scientific publications on the histopathology of canine parvoviral enteritis.[30] In time, the growing collection of surgical and autopsy specimens from MWDs at the AFIP allowed pathologists, epidemiologists, and clinicians from the AFIP and the DoD MWD Veterinary Service at Lackland Air Force Base to study and report on the lifetime occurrence of neoplasia and the causes of death, discharge from service, and euthanasia within large cohorts of MWDs.[28,31,32]

The Registry, as a concentrated source of MWD diagnostic case material, proved not only useful in studying MWD disease for the sake of MWD health and program management, but also as a source of information that could be extrapolated to the human veterans who accompanied MWDs on their deployments worldwide. Because MWDs and their American handlers shared similar physiologies and environmental exposures, MWDs became regarded as valuable sentinels for human disease.

One example that illustrates how MWDs can serve as sentinels of human disease is found in studies of MWDs that served in Vietnam. The observed increased risk of both seminoma (a type of testicular neoplasm) and testicular dysfunction in MWDs that served in Vietnam corroborated evidence of decreased sperm quality in human veterans who served in Vietnam (ie, "unexplained" and "significant" decreases in sperm quality were observed in human studies conducted by the Centers for Disease Control and Prevention or CDC).[33(p1042)] This correlation further suggested that testicular neoplasia should be studied as a potential experience-related cancer in veterans.[33]

In another example, veterinary pathologists at the AFIP studied lesions in deployed and nondeployed MWDs to get a better understanding of "Gulf War Syndrome" in veterans who served in the 1991 Persian Gulf War. However, this study found no significant difference in relative risk of neoplastic disease, neurologic mortality, or peripheral nerve disease associated with deployment to Southwest Asia in that war.[34,35]

Like their canine counterparts, marine mammals play vital roles as military working animals and receive diagnostic pathology support through Army veterinary pathology channels. (See also Chapter 7, Marine Mammal Program.) Military veterinary pathologists have made several key contributions to the body of knowledge of marine mammal pathology. For example, military veterinary pathologists studied and reported extensively on dolphin morbillivirus, considered the most important infectious disease of dolphins, responsible for at least two major epizootics resulting in large die-offs in US waters and, thereby, of significance to the Navy dolphin program.[36–40] Lieutenant Colonel (Retired) Thomas P. Lipscomb (written communication, 2012), who studied the disease extensively during his years in the AFIP Department of Veterinary Pathology, enlisted the assistance of Jeffrey K. Taubenberger, Chief of AFIP's Department of Molecular Pathology, to develop a polymerase chain reaction assay for morbillivirus to facilitate his studies of the dolphin epizootics.

Years later, Taubenberger and his team successfully sequenced the genetic code to the 1918 Spanish influenza, the influenza virus that killed over 50 million people worldwide in less than one year. The team used a specimen in the National Tissue Repository from a 21-year-old private who died in 1918 after being infected,[41] and Taubenberger later remarked that his experience using polymerase chain reaction to detect and sequence viruses from highly degraded dolphin specimens had proven very useful in the influenza study.

Other sea mammal and wildlife studies have also broadened the knowledge base of veterinary pathology. Lipscomb and colleagues at the AFIP characterized the pathology of genital carcinoma, a major disease of California sea lions and discovered a previously unknown gammaherpesvirus associated with the disease.[42] In the aftermath of the 1989 Exxon Valdez oil spill in Prince William Sound, Alaska, veterinary pathologists from the AFIP rotated through Seward, Alaska, at intervals to study the effects of the oil spill on coastal wildlife.[43] Their work was not only vital to the US Fish and Wildlife Department's investigation, but also resulted in the discovery of a novel herpesvirus infection in northern sea otters.[44]

Diagnostic achievements by military veterinary pathologists are often measured by the number of scientific publications they produced, but these pathologists have also produced other substantial written contributions to the profession. For instance, the AFIP, under the auspices of the American Registry of Pathology, published the series of fascicles that make up the *World Health Organization International Classification of Tumors of Domestic Animals*, a reference standard for neoplastic diagnoses in veterinary pathology. Leon Z. Saunders, who was assigned to the AFIP as a veterinary officer in the US Air Force Reserve from 1954 to 1964, authored the exhaustive historical text on veterinary pathology, *A Biographical History of Veterinary Pathology*.[12]

Figure 15-2: Pathology of Laboratory Animals course participants, 1957.
Photograph courtesy of the National Museum of Health and Medicine archives.

Also among the prolific written contributions to the specialty are the works of the late Colonel Floris M. Garner, head of the Department of Veterinary Pathology from 1964 to 1972. Garner, who led a platoon in the Normandy Campaign of World War II in 1944 and received a Purple Heart after being severely wounded by mortar fire in Conde'-sur-Vire, earned his DVM degree in 1950 from Washington State College.[45] He began residency training at the AFIP in 1958 under the tutelage of Charlie N. Barron, who is recognized for his work as an ACVP president and *Veterinary Pathology* journal editor.[46] Garner oversaw the training of some 40 residents, directed the Pathology of Laboratory Animals course (Figure 15-2), coordinated the activities of the World Health Organization's International Reference Center for Comparative Oncology, and served as president of the Washington, DC, Veterinary Medical Association. He later also served as president of the ACVP. Besides publishing some 90 scientific papers and various book chapters, he edited two textbooks, including the two-volume *Pathology of Laboratory Animals*.[45]

Projected Future of the 64D

The diagnostic contributions of 64Ds will continue to impact not only the veterinary pathology community, but also the military medical community at large. For example, the National Tissue Repository,

now in the custody of the JPC, is the largest of its kind in the world, with over 7.4 million cases, including some 32 million tissue samples and 55 million glass histopathology slides, dating back to 1917. Branded a "national treasure" by researchers worldwide, the repository contains approximately 100,000 veterinary cases, including those submitted to the Registry of Veterinary Pathology throughout its existence, making it a valuable collection for retrospective study.[47]

Expertise in diagnostics will remain a strength of 64Ds for the predictable future for several other reasons. Diagnostics are important to the work conducted in the DODVPR, the standard and virtually the sole means by which today's 64Ds are generated. Following this residency, Army veterinary pathologists on staff at the JPC use diagnostic proficiency to mentor and develop other residents in training. The JPC staff also provides important diagnostic services for government-owned and privately owned animals and for second-opinion diagnostic cases from civilian and military veterinary pathologists worldwide, in exchange for case material on which to train residents.

Moreover, the veterinary pathologist assigned to the veterinary laboratory in Landstuhl, Germany, is responsible not only for direct diagnostic support to MWDs and privately owned pets throughout the European Command (EUCOM) and Central Command (CENTCOM) areas of responsibility, but also supports the key mission of rabies diagnostics for CENTCOM, providing a critical link to public health. Even the 64Ds assigned to research institutions (ie, the vast majority of the area of concentration) must rely on their strength in diagnostic pathology to support the colony health of their research animals and effectively sustain and conduct research.

BIODEFENSE AND BIOMEDICAL RESEARCH

Definitions and Scope of Biodefense and Biomedical Research in Veterinary Pathology

A zoonotic disease is defined as an illness caused by an etiological agent capable of moving between species and is typically used to describe infection from a veterinary species to a human. Transmission from humans to animals is occasionally called reverse zoonosis or anthroponosis. In an evaluation of over 1,400 entities known to cause human disease, 61% were considered to be zoonotic.[48] Furthermore, the majority of pathogens that have been weaponized as potential terrorist biowarfare agents are classified as zoonoses, making them as much the domain of the veterinarian as of the medical community. Critically, many of these agents are common within veterinary species, or are at least studied intensively in the veterinarian's training, while the same disease, although virulent in humans, may have only received a cursory treatment in a medical doctor's training due to the rarity of infection.

At present, 47 diseases considered foreign to the United States are listed in the US Animal Health Association's foreign animal disease compendium, known as "The Gray Book." Many of these diseases are considered zoonotic, and at least four are listed as potential biological weapon agents.[49] (See also Chapter 11, Zoonotic and Animal Diseases of Military Importance.)

The concept of biodefense in protecting the United States against such diseases and agents is multifaceted. A terrorist attack on US domestic soil with a foreign animal disease could be as obvious as a detonated explosive device or be as dangerous as the covert introduction of foot and mouth disease into cattle populations at one of the many feedlots that dot the Midwest's rural landscape. The former would have a known and horrific, though limited, effect. The latter could potentially spread more insidiously, yet have an economic impact magnitudes greater than that of the local effects of a bomb. This kind of bioterrorist or agroterrorist attack would not only affect every American who eats food, but could also cost billions of dollars to halt or control.

Histopathology, reinforced by laboratory techniques that allow for more specific identification of a viral or bacterial agent, often remains the "gold standard" for rapid diagnostics of any animal disease outbreak, be it an emerging disease or a maliciously introduced pathogen. The veterinary pathologist is therefore often the first line of defense in the diagnosis and eventual control and eradication of a zoonotic disease.

History is filled with examples of the use of pathogens as weapons. In 1346, the Mongol army hurled plague-infested corpses over the walls of the Crimean city of Caffa. Some speculate that this early biological attack precipitated the Black Death, which eventually killed one-third of Europe's population.[50] During the French and Indian War of 1763, the British used smallpox-infected blankets to infect Native Americans in an effort to turn the tide of battle.[51] During World War I, German secret agents in the United States infected Allied horses bound for Europe with anthrax and glanders in an unsuccessful attempt to cripple them prior to their shipment into the European theater.[51] Additionally, in World War II, the Japanese physician Ishii and veterinarian Yujiro extensively tested and used bioweapons on the Chinese populace.[51]

The threat of such weapons is no less serious today. The US military and civilian leadership were reminded of this fact in the early 1990s after Soviet defectors revealed the enormous bioweapons program in the former Soviet Union. The use of anthrax-laced letters as terror weapons during several weeks in September 2001 further elevated the nation's biodefense awareness and reinforced research efforts. These letters contained *Bacillus anthracis* spores, the dormant stage of anthrax, and were handled in or mailed to various locations in Washington, DC; Boca Raton, Florida; and New York, New York.[52] One letter intended for a US senator was misdirected and ended up in a Sterling, Virginia, post office. According to the CDC, 22 people were infected by this attack, with 11 developing the highly lethal inhalational form of anthrax. Of these 11 victims, 5 died of the disease while the remaining 6 survived due to aggressive medical intervention.[53]

As the United States enters the second decade of its struggle against terrorists, the role of the veterinary pathologist conducting biomedical research to protect service members continues to be a priority for the DoD. The same research that directly protects the deployed soldier ultimately plays a larger role in global civilian health; in fact, the vaccines, therapeutics, and diagnostic tests designed to protect soldiers often have far-reaching public health benefits. For instance, development of a vaccine to prevent malaria morbidity and mortality in military personnel will also benefit the approximately 220 million people worldwide affected by this disease every year.[54] Development of a treatment for cutaneous leishmaniasis will not only allow successful treatment of forward-deployed soldiers exposed to this disease, but also will benefit the 1.5 million people diagnosed with the debilitating disease every year throughout the world.[55]

The terrorist attacks of September 11, 2001, and continuing terrorist attacks have also heightened the recognition of, and underscored the importance of, the veterinary pathologists' role in using animal models of disease in biodefense and biomedical research. Since these occurrences, myriad research projects have been conducted to improve the efficacy of currently available vaccines, therapeutics, and diagnostic tests for many biological agents, including anthrax,[56] as well as naturally occurring outbreaks of biomedical importance such as those caused by malaria.[57]

Because many highly virulent human diseases occur naturally in animals, veterinary pathologists play a key role in developing medical defenses and in elucidating animal models. These models of disease are critically important in understanding the pathogenesis of all zoonotic diseases. Animal models also represent the cornerstone of viable vaccine and antibiotic development because they provide an essential means of efficacy and side-effect testing of potential treatments prior to their use in humans. The remaining sections of this chapter provide more information about the veterinary pathologists' roles in biomedical research, the key centers of pathology research, the history of this specialized field, the major contributions of its many pioneers, and the basic methodology of the US Army's biodefense and biomedical research programs.

Military Institutes of Biodefense and Biomedical Research

In the past, most research on biological weapons was conducted solely by the military for a variety of reasons. First, the majority of pathogens employed as biologic warfare agents are seen only sporadically in nature and are not typically important chronic diseases. Therefore, they are not pervasive in the environment and do not provide a lucrative financial marketing incentive for most civilian pharmaceutical research.

Second, working with these agents requires maximum biocontainment facilities; the lofty costs associated with construction and maintenance of these facilities are often prohibitive for private businesses. Third, since dealing with these agents is extremely dangerous, the handling of these deadly pathogens has traditionally been the realm of military research scientists. In fact, military veterinary pathologists conduct perhaps the most dangerous work of all scientists who labor in biosafety level-4 suits. Because biocontainment suits are made of a soft plastic, they provide little protection to the military veterinary pathologists who must work with sharp instruments and handle jagged bones when conducting necropsies.

Despite these obstacles, some changes may be imminent. Total US public and private funding for biomedical research increased from $75.5 billion in 2003 to $101.1 billion in 2007.[58] Funding for directed biodefense research, as well as the number of federal and civilian institutions that conduct biodefense research, also has skyrocketed in recent years. A recent review of research dollars dedicated to this field revealed that US government civilian biodefense funding increased from $633.4 million in 2001 to $6.5 billion in 2011.[59] This marked augmentation in funding reveals a definite shift in the control over biodefense research. However, by virtue of longevity and technical expertise, the military remains the dominant group at the forefront of both biomedical and biodefense research.

The US Army Medical Research and Materiel Command (MRMC) provides management oversight for several key military research laboratories and holds the mandate for medical research, development, acquisition, and medical logistics management.[60] Two

military institutes in the DoD dedicated to biodefense and biomedical research, respectively, are the US Army Medical Research Institute of Infectious Diseases (USAMRIID) at Ft Detrick, Maryland, and the Walter Reed Army Institute of Research (WRAIR) in Silver Spring, Maryland.

United States Army Medical Research Institute of Infectious Diseases

Biodefense research in the United States did not begin in earnest until 1941 when the Secretary of War Henry L Stimson asked the National Academy of Sciences to conduct a threat assessment of biological weapons.[51] The investigative panel confirmed the feasibility and gravity of the threat, prompting President Franklin D. Roosevelt to initiate the War Reserve Service with the mission to study and produce medical countermeasures to biological weapons. Camp Detrick, a nondescript National Guard base in western Maryland about an hour north of Washington, DC, was chosen as the site where this bioweapons research facility could be built.[51] Many years later, in 1969, USAMRIID was established at Camp Detrick to conduct basic research on some of the world's most threatening diseases with potential use as biological weapons. With several high-level containment facilities, USAMRIID remains one of only a few laboratories globally that is capable of developing vaccines, therapeutics, and diagnostics to combat emerging biowarfare threats.

Initially, the bioweapons program focused on research directed toward the development of defensive countermeasure; the start of the Korean War changed this focus for a period. Because of potential use of biologic weapons by the North Koreans, Chinese, and Soviets in the Korean War, the United States felt compelled to intensify research into both offensive and defensive bioweapons.[51] The United States even went as far as developing offensive biowarfare agents in the 1950s and early to mid-1960s, but the military has never used them.[51]

The aggressive period of US research was short lived. On November 25, 1969, during a visit to Ft Detrick, President Richard M. Nixon publicly announced a halt to all offensive research into biologic weapon development, asserting that all US work with biologic agents would be strictly defensive in nature. President Nixon also affirmed that all biodefense efforts would be devoted solely to producing vaccines, therapeutics, and diagnostics to detect and fight biowarfare agents, not to propagate them.[51]

In 1972, the United Nations enacted the Biologic Weapons Convention which mandated an end to any offensive, malicious, or otherwise deleterious research on biologic agents.[51] Among the initial signatories in 1972 were the United States and the former Soviet Union.

Emphasis on defensive research of biological agents has led to public health advances in the United States and globally. Over the past 30 years, USAMRIID pathologists have made significant and timely contributions to the detection and response of disease outbreaks. One example is the 1989 outbreak of hemorrhagic fever that swept through a colony of research monkeys at a nonhuman primate quarantine facility in Reston, Virginia, less than 15 miles outside of the Washington, DC, beltway. Suspecting a deadly virus as the causative agent, the veterinarian employed to manage the colony contacted USAMRIID for assistance.[61]

USAMRIID researchers isolated and identified the virus as a new species of Ebolavirus that had never before been described in any animal species or humans (Figures 15-3a and 15-3b). The novel virus was named Ebola Reston in recognition of the location of the first confirmed outbreak of the agent. A US Army veterinary pathologist, Colonel (Retired) Nancy Jaax, played a role in the diagnosis of the disease by performing necropsies and pathologic analysis on several of the stricken monkeys. Her analysis also helped characterize and confirm the virus presence and subsequently led to efforts that halted the spread of the newly discovered virus.[61] (See also Chapter 1, Military Veterinary Support Before and After 1916, for a more complete story about the discovery of this novel virus and other veterinary contributions to public health.)

Ten years later, in 1999, another puzzling disease raged, this time in New York City, causing widespread, sporadic deaths of various species of birds, horses, and even humans in its wake. Initially, medical officials attributed the human disease cases to St Louis encephalitis virus, a disease spread by mosquitoes with historically sporadic outbreaks occurring within the United States.[62]

However, Dr Tracey McNamara, a veterinary pathologist at the Bronx Zoo, noted the alarming rate of bird deaths occurring during the fatal human outbreak and reached a different conclusion. She contacted the CDC with her theory that the disease outbreak was not due to St Louis encephalitis because this disease typically did not affect birds. McNamara believed the avian and human deaths were linked and were more likely caused by another deadly arthropod-borne virus, but she did not have the diagnostic capabilities to determine the cause. When researchers at the CDC dismissed her thoughts that the human deaths in New York City were caused by an "animal" virus, McNamara reached out to military veterinary pathologists at USAMRIID.

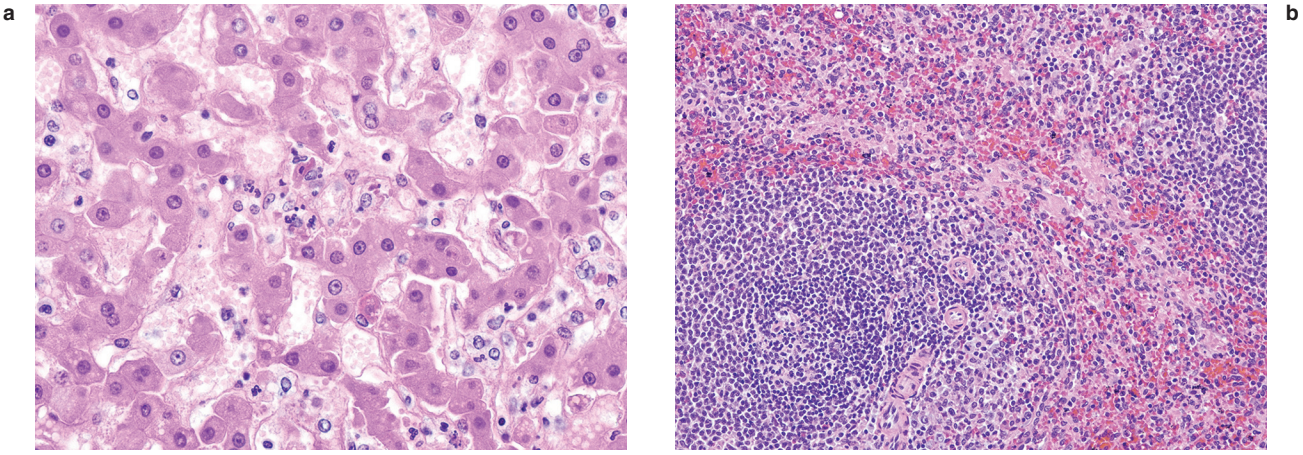

Figure 15-3: Lesions from Ebola virus. Liver and spleen from a rhesus macaque (Macaca mulatta) experimentally infected with Ebola virus. (a) Liver: Multifocal necrosis of hepatocytes with rare, eosinophilic, intracytoplasmic inclusions, HE 400×. (b) Spleen: Diffuse deposition of fibrin within the red pulp with lymphoid depletion in the white pulp. HE 200x. Photomicrographs courtesy of Major Todd Bell, US Army Veterinary Corps, US Army Medical Research Institute of Infectious Diseases, Frederick, MD.

McNamara provided tissue samples from several of the dead birds (from the wild and from the Bronx Zoo collection) to military veterinary pathologists and virologists at USAMRIID, and they used polymerase chain reaction assay to identify the causative agent as West Nile virus (WNV). Transmitted by mosquitoes, WNV is an arthropod-borne virus closely related to St Louis encephalitis; this was the first time WNV was detected as a human pathogen in the United States.[62]

Following this discovery, several federal agencies and the CDC labs confirmed the diagnosis. However, the initial work done at USAMRIID was the corroborative evidence needed to definitively prove that WNV was indeed the culprit in this viral mystery (K Steele, personal oral communication, April 24, 2012). The identification of the disease allowed the public to be alerted and informed, helping mitigate the effects of this emerging viral threat. In subsequent scientific work, USAMRIID scientists collaborated to further characterize the virus.[62]

Emphasis on defensive research of biological agents for disease protection is not USAMRIID's only current mission. The release of the anthrax letters on the heels of the September 11, 2001, attacks prompted a review of America's preparedness to deal with biologically guided terrorist attacks. From this review was born the National Interagency Biodefense Campus initiative, designed to increase collaboration of basic infectious disease research as well as expedite development of diagnostic assays, vaccines, and therapeutics. USAMRIID, as representative of the DoD; the National Institute of Allergy and Infectious Diseases (NIAID), of the National Institutes of Health; and the National Biodefense Analysis and Countermeasures Center, of the Department of Homeland Security, collaborate jointly in this initiative. Their co-location on the Ft Detrick campus underscores the cooperative efforts to protect service members and civilians from infectious disease and to safeguard the nation from biological attack.

The Walter Reed Army Institute of Research

The WRAIR, originally named the Army Medical School, was founded in 1893 by US Army Surgeon General George Sternberg.[63] The WRAIR adopted its current title in 1955 and is named after Major Walter Reed, the pioneer in biomedical research who provided proof that yellow fever was spread by a mosquito vector. The WRAIR currently conducts biomedical research primarily focused on health and readiness to ensure that America's service members are equipped with the most effective medical defenses and treatments against international health threats.

More specifically, the WRAIR conducts research on a range of militarily relevant matters, including operational health hazards, combat casualty care, and naturally occurring infectious diseases. It is a lead agency for infectious disease research through basic science and clinical research and a crucial source of research for medical product development. Because of its pioneering focus on disease prevention, the WRAIR is widely recognized as the oldest public health and preventive medicine institute in the United States. It is also the oldest subordinate laboratory of the MRMC and the largest biomedical research institute within the DoD.

The WRAIR hosts two centers: The Center for Military Psychiatry and Neuroscience and the Center for Military Infectious Disease Research. The Naval Medical Research Center, co-located with the WRAIR on the Forest Glen campus in Silver Spring, has an Infectious Disease Directorate and an Operational and Undersea Medicine Directorate. The Department of Pathology, which employs five military veterinary pathologists, provides research support for both the WRAIR and the Naval Medical Research Center. This department's research support focuses on prevention and treatment of diseases and conditions relevant to the current operational environment, including blast exposure and traumatic brain injury; biomarkers to detect evidence of traumatic brain injury; animal model development to replicate posttraumatic stress disorder; vaccines and drugs for prevention and treatment of infectious diseases such as malaria, HIV/AIDS, dengue fever, and leishmaniasis (Figures 15-4a and 15-4b) and enteric diseases; and animal model development to improve treatment of decompression sickness. With current and ongoing operations in the Middle East, new research focuses on wound infection healing and treatment; medical countermeasures for multidrug-resistant organisms and their effect on wound healing of amputees; treatments for hemorrhagic shock; improved hemostatic dressings; and treatment of ischemia/reperfusion injury.

The research and development activities of the WRAIR extend worldwide, wherever disease agents that pose a threat to deployed US forces are endemic. WRAIR has four subordinate laboratories located on three continents where clinical trials are conducted and products are developed and tested to detect, control, and prevent infectious diseases of strategic significance to the US military: US Army Medical Component-Armed Forces Research Institute of Medical Sciences (USAMC-AFRIMS); the US Army Medical Research Unit-Kenya (USAMRU-K); the US Army Medical Research Unit-Europe (USAMRU-E); and the US Army Medical Research Unit-Georgia (USAMRU-G).

USAMC-AFRIMS, in Bangkok, Thailand, and USAMRU-K, in Nairobi, have been integral in developing and testing improved means for predicting, detecting, preventing, and treating diseases such as malaria, infectious diarrhea, and HIV/AIDS. USAMC-AFRIMS and USAMRU-K also conduct surveillance, training, research, and response activities related to emerging disease threats. Additionally, these units provide regional support, enable capacity building, and nurture long-standing relationships with other militaries and governmental organizations.

Located in Sembach, Germany, USAMRU-E studies diseases and conducts applied psychological research to protect, optimize, and enhance soldier psychological resilience, including support and evaluation of the Army's mental health advisory teams, more commonly known as MHATs. The newly established USAMRU-G, located in Tbilisi, focuses on endemic disease research, public health, and disease surveillance, as well as providing regional support and capacity building.

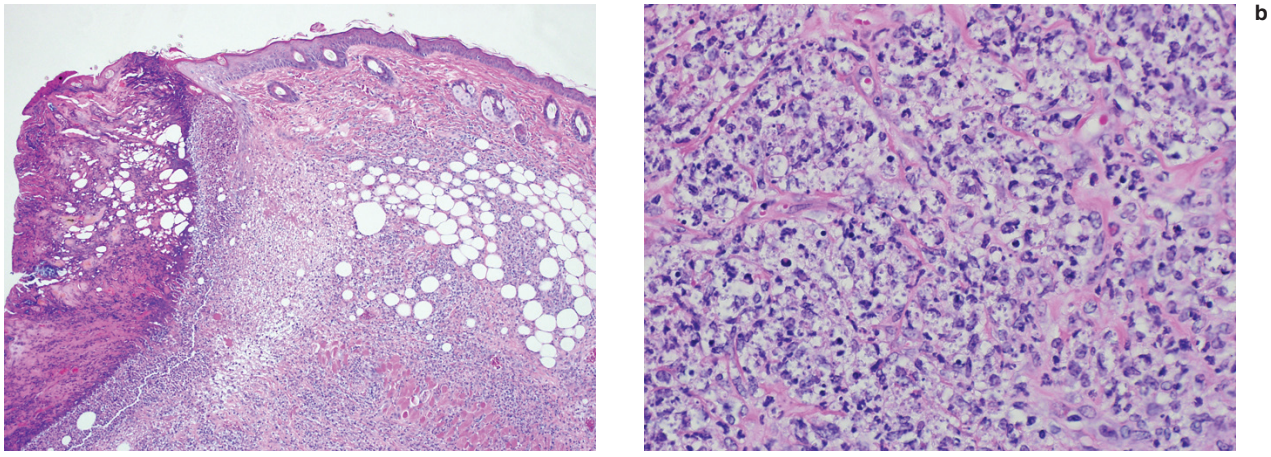

Figure 15-4: Cutaneous leishmaniasis. Haired skin from a BALB/c mouse experimentally infected with Leishmania major. **(a)** Necrosis and ulceration of the epidermis with replacement by a thick serocellular crust. The dermis and subcutis is markedly expanded by inflammatory cells, fibrin, and edema. HE 100X. **(b)** Higher magnification of inflammation in the dermis. Numerous macrophages are present and filled with intracellular amastigotes. HE 600X.
Photomicrographs courtesy of Lieutenant Colonel Jennifer Chapman, US Army Veterinary Corps, Walter Reed Army Institute of Research, Forest Glen, Maryland.

Prioritizing Major Emerging Threats to Civilian and Military Populations

How does the DoD decide what pathogens should be studied for biodefense purposes, and by extension, which biological agents will be targeted for vaccine and therapeutic development? Usually, the DoD develops its strategic plan from a classified military perspective after considering input from allied civilian agencies. For instance, the DoD, in collaboration with the US Department of Health and Human Services (HHS), the US Department of Homeland Security, and other federal agencies set biodefense research priorities based on one of two lists. The first is the HHS and US Department of Agriculture (USDA) Select Agents and Toxins, a list of biologic agents and toxins defined as having the potential to cause a grave threat to public health, plant health, or animal and plant products.[60] The second list, generated by NIAID, is referred to as the NIAID Category A, B, C Priority Pathogens list.[61]

The impetus for forming these lists was a 1995 event in which a rogue microbiologist was able to purchase a stockpile of *Yersinia pestis*, the causative agent of plague, through the US Postal Service.[60] At that time, no pertinent rules or regulations guided the transport of these agents in the United States. The US Congress responded with Section 511 of the Antiterrorism and Effective Death Penalty Act of 1996 (Public Law 104-132), which requires (*a*) the maintenance of lists of biologic agents and toxins with the potential to endanger public health; (*b*) the development of a system to govern the movement of these selected pathogens; and (*c*) the training necessary for individuals to work with or transfer these pathogens. The HHS turned to the CDC to administer this new program, and the CDC established the Select Agents and Toxins program for this purpose.[60]

The CDC uses the following six criteria to decide which agents are included on the HHS and USDA Select Agents and Toxins list: (1) virulence, pathogenicity, or toxicity of the agent; (2) availability of treatment (ie, vaccines, antibiotics, antitoxins, or other treatments); (3) transmissibility of the organism; (4) technical difficulty in reproducing or growing the organism; (4) ease of dissemination; (5) potential to cause public panic; and (6) known research and development by a state sponsor.[59] Agents that are highly virulent, have no known treatments, are easily transmitted, and can be easily grown or reproduced in a laboratory are of greatest concern.

In the NIAID Category A, B, and C Priority Pathogens list, Category A pathogens are the highest priority and are those agents that create the maximum risk to national security and public health because they (*a*) can be easily disseminated or transmitted from person to person; *(b)* result in high mortality and have the potential for major public health impact; *(c)* might cause public panic and social disruption; and *(d)* require special action for public health preparedness. Category B pathogens are the second highest priority and are those agents that *(a)* are modestly easy to disseminate; *(b)* result in modest morbidity rates and low mortality rates; and *(c)* require specific enhancements for diagnostic capacity and enhanced disease surveillance. Category C pathogens are those that are the third highest priority and include emerging agents that could be engineered for mass dissemination in the future because of *(a)* availability; *(b)* ease of production; and *(c)* potential for high morbidity and mortality rates and a large health consequence.[64]

The HHS and USDA Select Agents and Toxins list and the NIAID Category A, B, and C Priority Pathogens list are often confused. Significant overlap exists between these two lists, but NIAID's is the most comprehensive. However, if an agent or pathogen is on either of these lists, it is a dangerous microbe for which the United States needs viable treatment modality available to protect citizens and military personnel.

Unlike public biodefense research, biomedical research in the DoD focuses more on diseases of military significance and is guided by analysis and risk assessment of infectious disease threats to deployed US forces. Therefore, the National Center for Medical Intelligence (formerly the Armed Forces Medical Intelligence Center) has defined infectious diseases of military significance based on the following criteria: *(a)* the disease is capable of degrading military operations; *(b)* the disease is severe; or *(c)* the disease has, historically, been a force health protection concern for commanders.[65] This list is reevaluated regularly to capture new or emerging diseases that have become established. The top three endemic disease threats, as defined in a 2008 study, are malaria, bacterial diarrhea, and dengue fever, all of which are priorities in DoD biomedical research.[65]

Understanding the Research Methodology of the Veterinary Pathologist

Research involving infectious microorganisms, especially those involved in biodefense activities, is conducted under strict guidelines, and the necessary biocontainment levels to conduct such research are set based upon the risk of a given agent. These biosafety levels are set by the CDC and published in the Biosafety in Microbiological and Biomedical Laboratories document. The standard biosafety levels in ascending order are biosafety level 1 or BSL-1, BSL-2, BSL- 3, and BSL-4.[66] BSL-1 agents are well-studied and understood agents that do not commonly or consistently cause disease in healthy adults and are deemed a negligible

hazard to those working with them. BSL-2 agents are considered a moderate hazard to those working with them. BSL-3 agents can cause serious or potentially fatal disease if personnel are exposed to them, but are agents for which there is a known treatment. BSL-4 agents are those that can cause severe disease or death and for which no known treatment is available.[66]

USAMRIID conducts research primarily on BSL-3 and BSL-4 agents, whereas WRAIR conducts research on agents in BSL-1 and BSL-2. Much of the DoD work conducted with biological agents on either the HHS and USDA Select Agents and Toxins list or the NIAID Category A, B, and C Priority Pathogen list is conducted in either BSL-3 or BSL-4 labs at USAMRIID. Work in biocontainment with agents that could be life-threatening is both physically and mentally taxing. As noted earlier, veterinary pathologists regularly put their lives at risk performing animal necropsies in biocontainment to advance research needed to develop medical countermeasures for the nation's protection (Figure 15-5).

Once an emerging virus or other pathogen is identified for scientific research, several questions must be answered. How does the pathogen infect the host under typical natural conditions to cause disease? What physiological mechanisms does the pathogen use to cause disease in the host? What vulnerability can be exploited to allow a vaccine or therapeutic modality to be successful in combating disease or protecting an individual from infection?

Since the majority of diseases on the HHS and USDA Select Agents and Toxins list are zoonoses, the veterinary pathologist is a key subject matter expert when conducting research to answer such questions. Much basic disease research (ie, to elucidate pathogen behavior within a host and combat its effects) has been conducted at both the WRAIR and USAMRIID since the 1940s; veterinary pathologists have been instrumental to this research, contributing critical information establishing the infectious dose of a particular pathogen, the lethal dose required, the variability in disease manifestation depending on the route of exposure, and the pathogenesis of numerous disease-causing organisms.

The following is an oversimplified description of the process to develop a medical countermeasure for a disease with no existing drug, vaccine, or other therapy; this explanation is meant to give the reader merely the essentials of how the process is conducted. First, basic research is performed to characterize the pathogen and identify potential vulnerabilities in the organism's physical or molecular structure that may serve as a means of attacking or preventing disease. The physiological mechanisms involved during infection of the host are then characterized and identified in an attempt to either capitalize on the host's defenses against the organism, augment the host's defenses to overcome infection, or possibly even mitigate a host's immunological or inflammatory response to lessen the deleterious effects of inflammatory mediators in the host.

Once a potential medical countermeasure is deemed a viable candidate for further investigation, initial or "preclinical" testing is initiated. If the treatment shows promise by protecting tissue culture cells in a petri dish from pathogen challenge, small-scale testing in a progression of laboratory animal models is generally the next step, with the studies methodically advancing from small rodents through other animal species that have been developed and identified as appropriate models to more closely replicate the physiological response of humans. Safety, dosing, carcinogenicity, efficacy, and immunogenicity studies are performed in the preclinical phase; also, during this phase, a standard and repeatable method of producing the vaccine or treatment is established to ensure a quality product (ie, Good Manufacturing Practices). The veterinary pathologist is integrally involved in key aspects of this process, most crucially in analyzing and interpreting lesions caused by the disease, determining which lesions may be a result of the treatment modality and which lesions are neither pathogen-related nor treatment-related.

When efforts have proven successful and promising to this point and the treatment or vaccine is safe and efficacious in animal models against the disease of interest, the preclinical data is submitted to the US

Figure 15-5: A military veterinary pathologist and military research technician work as a team to collect tissue specimens during a necropsy in a BSL-4 suite at the US Army Medical Research Institute of Infectious Diseases.
Photograph courtesy of Major Todd Bell, US Army Veterinary Corps, USAMRIID, Frederick, MD

Food and Drug Administration (FDA) for an investigational new drug approval. All study data collected to this point is closely scrutinized for strict adherence to Good Laboratory Practices requirements. All parts of the study, from the housing and care of the animals to the paperwork documenting the study findings, are meticulously examined. Once investigational new drug approval is obtained, small-scale, limited testing of healthy, human volunteers can begin.

Phase I clinical trials are defined as testing in limited groups of healthy adults to gather safety and immunogenicity data. Phase II clinical trials are defined as testing in larger groups to continue to refine the safety, efficacy, and dosing information.[59] Phase III testing is conducted in larger groups of people who are affected with the disease being studied or are likely to be exposed to the disease being studied (eg, the testing of anti-malarial drugs in people residing in malaria-endemic areas). If results are favorable at each step, overall approval is sought from the FDA.[59] If at any stage serious adverse events are encountered, the study is immediately halted, and researchers return to prior steps to reexamine the medical treatment regimen. (See Table 15-1 for trial or testing phase definitions and objectives.)

TABLE 15-1

THE THREE PHASES OF CLINICAL TRIALS

Phase	Participants	Objective
Phase I	Limited group of healthy adults	Safety and immunogenicity data
Phase II	Larger group of healthy adults	Safety, efficacy, dosing
Phase III	Very large groups of people affected by the disease or likely to be exposed to the disease	Safety, efficacy

The medical countermeasure development process is rife with pitfalls and stumbling blocks, and false starts are common. The general timeframe from initial early research and development of potential treatment candidates to successful product development and FDA approval is over 10 to 15 years. The process may require about $800 million to $1 billion in capital outlay, with a very high likelihood of failure at any point. To confound this process further, in the case of military medicine and biodefense, the majority of agents studied in this field have the potential to cause severe disease or death in humans, morally precluding the standard use of human clinical trials, and there is no commercial market or alternative use for the medical countermeasure.

Because of these constraints, development of appropriate animal models that mimic the human course of disease is crucial to understanding the effects of biological warfare agents. Recently, at the insistence of the DoD and the biodefense community, a new FDA requirement was instituted (informally termed the "animal rule") that requires the use of at least two appropriate animal model species to establish the safety and efficacy of products against biological warfare agents.[67] The animal rule allows for the development of human treatments and vaccines based on animal models of disease when human testing would be either unethical or simply infeasible.

CHEMICAL DEFENSE

Overview of Chemical Defense Resources, Duties, and Roots

The US Army Medical Research Institute of Chemical Defense (USAMRICD), located at the Aberdeen Proving Ground-Edgewood, Maryland, is the federal resource tasked with product development, testing, and evaluation against a growing array of chemical threats to both soldiers in the field and to civilian responders in the United States.[68] USAMRICD is the DoD's leading laboratory for medical chemical defense research and assists in formulating policies and medical doctrine related to traditional and nontraditional chemical warfare agents. To aid USAMRICD's mission, the military veterinary pathologist provides a greater understanding of the pathological, biochemical, and toxicological consequences of exposure to chemical warfare agents and in the assessment of the efficacy of medical therapeutics and countermeasures.

USAMRICD's roots stem from the US Army's Chemical Warfare Service (CWS), precursor to the Chemical Corps; the CWS was established in 1917 with seven main divisions, each focused on different areas: (1) research; (2) training; (3) development; (4) proving ground; (5) gas defense; and (6) medical. Originally, the seventh division, the gas offense division, had its main facility on Edgewood Arsenal, just north of Baltimore, Maryland, and the current location of USAMRICD. In 1919, this arsenal was the center of training, stockpiling, and research and development of chemical warfare agents for the US Army.[69]

The medical division can trace its origins to the AMEDD and was responsible for the pharmacological aspects of medical defense against chemical weapons

and for the treatment of chemical weapon casualties during World War I. The division was reorganized in 1922 as the Medical Research Division at Edgewood Arsenal. In the early 1960s, this division was renamed the US Army Biomedical Laboratory. In 1979, command of the laboratory was assumed by the US Army Medical Research and Development Command (now the MRMC) when the Army surgeon general assumed command of all medical chemical defense. In 1981, the laboratory received its current name, USAMRICD; it is now one of six medical laboratories and research institutes under the command of MRMC.

Brief History of Chemical Threats

As early as 3000 BCE, ancient Egyptian and Indian civilizations cultivated, studied, and accumulated poisons from plants, animals, and minerals.[69] In broad terms, chemical warfare agents can be described as any substance or compound (natural or synthetic) designed, intended, and used for the purpose of killing, seriously injuring, or incapacitating others. Early chemical warfare agents typically were simple and used in conjunction with wooden projectiles, fast-moving metal projectiles, and incendiary devices to develop poisonous, noxious, or irritant vapors. Although advocates in both the Union and Confederate armies proposed using chemical warfare agents such as liquid chlorine, chloroform, hydrochloric acid, sulfuric acid, and Chinese "stink bombs" during the US Civil War, historically, chemical weapons have not actually been used in attacks on the American homeland.[69–72]

Modern chemical warfare began in other countries in World War I. The lengthy stalemates associated with trench warfare during this war directly correlated with increased technological advances of chemical warfare agents for battlefield use. France first used chemical agents (eg, ethyl bromoacetate or tear gas) in 1914 against the Germans, but this implementation was ineffective.[69] In April 1915, during the Second Battle of Ypres, Germany delivered the first successful chemical warfare attack against the Allies using toxic chlorine vapors projected from cylinders.[73–76] Later that year, Germany debuted the use of phosgene and diphosgene gases, followed by French use of hydrogen cyanide in 1916, and German use of chemical mustard gas in July 1917.[69] Mustard agent, feared most by American soldiers, caused 20,000 casualties in only 6 weeks after its introduction and ultimately debilitated over 27,000 Americans by the end of World War I.[69, 77]

In April 1917, despite an earlier position of strict neutrality, President Woodrow Wilson asked Congress for a formal declaration of war on Germany following increased German U-boat attacks on American merchant ships. Soon after, preparation for chemical warfare began, and the US Army's CWS was established with full responsibility for all facilities and functions relating to toxic chemicals. (In 1946, Public Law 607 changed the name of the CWS to the Chemical Corps.[78])

Between World War I and World War II, Italy employed chemical mustard agent during their invasion of Ethiopia in 1935, and the Japanese deployed an extensive chemical weapons arsenal, in addition to biological warfare agents, during their invasion of China in the late 1930s. During the interwar period, the first nerve agents (derived from the organophosphorus or OP compounds tabun and sarin) also were developed and evaluated by German scientists. Nerve agents were considered ideal weapons because of their colorless and odorless nature and deadly effects.[69,79,80] Despite an aggressive approach to the development of nerve agents as an offensive weapon, Germany's reluctance to use nerve gas during World War II remains an enigma. Nonetheless, Germany, Japan, Great Britain, and the United States did have active plans to use various forms of chemical weapons in the event opposing forces used them first.[69,80]

Although smoke, flame, defoliants, and nonlethal riot control agents were used in the Korean and the Vietnam wars, there is no evidence that the US Army Chemical Corps ever employed debilitating chemical weapons during either war.[69] Still, the United States continued its chemical agent production program until 1969. By then, a combination of growing public hostility, US involvement in the Vietnam War, use of riot control agents and defoliants in Vietnam and in the United States, and a series of high-profile chemical agent-related incidents at Dugway Proving Ground, Utah, and in Okinawa, Japan, caused President Nixon to effectively halt the production of chemical weapons in America.[69,81] Consequent plans to abolish the Chemical Corps entirely led to a temporary decline in the US chemical defense program in subsequent years.

However, interest has since increased for several reasons. The Arab-Israeli Yom Kippur War in 1973 and various skirmishes and wars in Southeast Asia and Afghanistan, directly (or indirectly) involving the Soviet Union throughout the 1970s and 1980s, strongly indicated that the Soviets were ready for, and potentially intended to use, extensive chemical warfare.[69] Iraq's use of chemical warfare against Iranian soldiers during the 1980s also strongly signaled that formulating a plan to not only restore, but increase, US defensive capability against chemical warfare agents was prudent. Although no known chemical or biological attacks were made by Iraqi forces during Operation Desert Storm in 1991, all deploying US military units were

fully equipped with the latest chemical and biological defensive equipment, and troops were administered prophylactic vaccines against anthrax and botulinum toxin. Additionally, pyridostigmine bromide tablets were dispensed as a nerve agent pretreatment, and the Mark I nerve agent antidote kit was issued to treat nerve agent poisoning.[69,82,83]

According to available US data, there was no known deployment of chemical weapons by insurgents during Operation Enduring Freedom in Afghanistan, which began in 2001, but there have been several documented cases of Iraqi insurgents using chlorine gas car bombs in Operation Iraqi Freedom in 2007.[69,84,85] Future terrorist chemical attacks on US service members and civilians, both domestically and abroad, probably will be similarly isolated in nature, unlike the full-scale chemical warfare seen during World War I. However, in 2013, United Nations chemical weapons inspectors, consisting of a team of nonpartisan scientific experts, confirmed that surface-to-surface rockets containing the chemical nerve agent sarin had been deployed between parties and against civilians, including children, in an ongoing civil conflict in the Syrian Arab Republic.[86]

Overview of Chemical Agents and Military Research

Chemical agents used to kill, seriously injure, or incapacitate victims are typically classified according to their physical state (ie, solid, liquid, or gas); physiological action; and use. A persistent or nonpersistent nature does not definitively classify a chemical agent, but is used to signify the time the chemical agent remains in the area. In general, chemical agents are categorized as follows: vesicants (ie, blister agents); pulmonary choking agents (ie, lung-damaging agents); cyanide; nerve agents; riot control agents; or incapacitating agents. Although riot control and incapacitating agents have been extensively studied in military medicine, military veterinary pathologists have focused their research on nerve agents, vesicants, pulmonary choking agents, and, to a lesser extent, cyanide.

Nerve Agents

Nerve agents, the most toxic of the known chemical agents, are OP compounds that exert their biological effects through inhibition of the enzyme acetylcholinesterase (AChE).[69,87] Originally produced during a search for ideal insecticides, OP compounds were evaluated for military use because of their toxicity. The five most common nerve agents of military interest are tabun (GA), sarin (GB), soman (GD), and the compounds simply designated as VX and GF. [88]

In the body's cholinergic nervous system, action potentials stimulate release of the neurotransmitter acetylcholine from presynaptic vesicles within the neuromuscular junction, resulting in the formation of postsynaptic action potentials that trigger a contractile response of muscle and glands. AChE, found at the synaptic receptor sites, rapidly hydrolyzes and terminates acetylcholine's activity. If AChE is absent, or altered, acetylcholine continues to stimulate the affected organ. Thus, clinical signs of OP nerve agent exposure include spasms, seizures, and/or hypersecretion in organs with cholinergic receptor sites, such as smooth and skeletal muscles, the central nervous system, and most exocrine sweat glands.

Vesicants

Three vesicant agents are of significance to the US military: sulfur mustard (HD), lewisite (L), and phosgene oxime (CX). Of the three, HD is the first and only vesicant known to be used as a chemical weapon on the battlefield.[88,89] Generally, lesions caused by lewisite and CX are less severe than those caused by HD. Additionally, unlike HD and lewisite which cause blisters, CX is not considered a true vesicant, but rather an urticant, since it causes dermal erythema and swollen red bumps or plaques (eg, wheals and hives) on the skin surface.[88,89]

Pulmonary Choking Agents

Pulmonary choking agents, also known as lung-damaging agents or pulmonary edematogenic agents, are generally separated based on their pathophysiology and where they cause damage within the respiratory tract. Although HD is considered a vesicant, it is also considered a central pulmonary agent.

Central pulmonary agents such as HD and ammonia form strong acids or bases within central airways where bulk air flow occurs (ie, in the trachea, bronchi, and bronchioles) and irritate or damage the tissues, particularly surface epithelial cells.[88,89]

Conversely, peripheral pulmonary agents (ie, edematogenic agents) affect the gas-exchange regions distal to the terminal bronchioles where bulk air flow is absent during each breath (ie, the respiratory bronchioles and alveoli). These agents typically cause pulmonary edema by damaging the endothelial lining of alveolar septa, resulting in accumulation of fluid in alveoli and bronchioles and pleural effusion.[88,90] Examples of peripheral pulmonary agents include phosgene (CG), perfluoroisobutylene (PFIB), oxides of nitrogen, and hexachloroethane (HC) smoke. In particular, PFIB, a product produced by the prolysis

of polytetrafluoroethylene (PTFE, brand name Teflon, Dupont, Wilmington, Delaware), causes respiratory flu-like symptoms called "polymer fume fever."[91,92] Some agents, especially at higher doses, will affect both central and peripheral respiratory compartments (eg, chlorine).

Cyanide

Cyanide intoxication occurs following ingestion, inhalation, or injection of hydrogen cyanide (AC) and cyanogen chloride (CK), and it produces death in humans within 8 to10 minutes following exposure.[75,76] Historically, cyanides have been termed "blood agents" although cyanide exerts its most pathogenic affects primarily outside the bloodstream, specifically in organs with high oxygen requirements and dependency on aerobic respiration (eg, brain, heart, and liver).[93,94] At the subcellular level, cyanide inhibits mitochondrial cytochrome oxidase, causing impairment of intracellular oxygen utilization and depression of cellular respiration.[94] In the central nervous system, the effects of cyanide toxicity are related to the direct effect on neurons with glutamic acid receptors.[94–96]

Military Veterinary Pathologists in Chemical Defense and Animal Model Development

Nonliving chemical agent models can be used as a screening tool to investigate mechanistic interactions and to down-select potential treatment options; however, they cannot model the complex interactions that occur in live models during the injury and repair phases of chemically induced injury. Therefore, appropriate animal models must continue to be researched, developed, and used to define various chemical injury mechanisms and classifications and to further develop preexposure and postexposure protectants and therapies.[85]

DoD research has focused on developing countermeasures against nerve and HD agents. Study of the lethal effects of nerve agent exposures started in early 1980 and culminated in November 1990 with the fielding of the anticonvulsant drug diazepam, packaged as Convulsant Antidote Nerve Agent (CANA) and intended for use as an immediate field treatment of nerve agent-induced seizures.[97] Army veterinary pathologists, teamed with USAMRICD investigators, played crucial roles establishing the nonhuman primate and rodent animal models used to define the basic neuropharmacological mechanisms of nerve agent-induced seizures and to characterize the neuropathology and cardiomyopathy lesions in survivors and nonsurvivors following nerve agent exposure.[97–104]

Hallmark lesions, particularly in soman- and sarin-induced toxicity, include myocardial degeneration and necrosis; neuronal degeneration and necrosis; and neuropil edema within the cerebral cortex, amygdaloid complex, hippocampus, and multiple thalamic nuclei. By 1987, additional studies clearly indicated that nerve agent-induced brain damage was primarily the result of prolonged seizure activity.[97,105] This critical discovery resulted in the addition of diazepam (ie, as CANA, an autoinjector containing 10 mg diazepam) or other benzodiazepine anticonvulsant drugs to the standard nerve agent medical therapy in order to minimize or prevent brain lesion development and to enhance survival following nerve agent exposure.[82,97,99,105–109]

Like nerve agent research, HD studies have focused on determining mechanisms of action and exposure-related pathologies to advance development of preexposure and postexposure treatments. Animal models used to study HD exposure to skin include the hairless guinea pig, weanling pig, and the mouse ear and hairless mouse.[97] From work on these animals, USAMRICD researchers and military pathologists have defined a sequential preblistering phase that develops following HD exposure. In the prephase, epidermal basal cells and basement membrane constituents are targeted, eventually resulting in microscopic blisters at the epidermal-dermal junction.[110–114]

The effects of HD exposure on eyes and airways have also been scrutinized. Eyes are most sensitive to HD-induced injury, and the pathogenesis of HD ocular lesions has been described in studies using light and electron microscopy on rabbits.[89,115,116] According to documented pulmonary studies, several sequential changes occur in airways following HD inhalation: first, necrosis of upper airway epithelium; next, lower airway necrosis and epithelial sloughing; and finally, obstructive pseudomembrane formation, an important cause of death in animals within the first 24 hours after exposure.[82,88,117,118] Exposed animals died primarily from pulmonary injury complicated by infection (eg, bronchopneumonia). Complications from HD-induced bone marrow suppression, hemorrhagic pulmonary edema, and pleural effusion have also been documented in cases of high-dose exposures.[88,114]

Nerve agents and HD are not the only chemical agents that veterinary pathologists have studied using animal models. Rats, mice, and rabbits have long been part of phosgene pathogenesis and treatment studies.[119–125] In fact, a mouse model was used by an Army veterinary pathologist and other USAMRCID investigators to first correlate histopathological acute phosgene-induced pulmonary injury to the presence of leukocytes in bronchoalveolar lavage fluid and elevations in serum protein and lactate dehydrogenase levels. Neurotoxic, cardio-

toxic, and hepatotoxic lesions have also been described for acute and long-term cyanide intoxication studies in a wide variety of animal models, including mice, rats, rabbits, cats, dogs, pigs, goats, and monkeys.[126–131]

Although US military forces have not engaged in chemical warfare since World War I, lessons learned from chlorine-laced car bombs utilized by Operation Iraqi Freedom insurgents reinforce current beliefs that military personnel must always be prepared and equipped to operate in any environment where chemical agents may be used. The Aum Shinrikyo's use of sarin gas to attack a Tokyo, Japan, subway tunnel in 1995 further underscores this sobering reality.[132–135] Even though this cult was targeting greater lethality, approximately 1,100 people presented with mild to severe clinical signs and symptoms of sarin poisoning from this city attack. The clear and convincing evidence of munitions containing sarin being used with lethal consequences on a relatively large scale in Syria in 2013 is also concerning. US military forces will continue to rely on research conducted at USAMRICD, with its wide array of scientific expertise, including veterinary pathologists, to ensure chemical preparedness on battlefields and in urban environments.

RADIATION DEFENSE

Development of the Radiation Program

In 1957—2 years after a controlled test explosion of a thermonuclear device in the Pacific Ocean transcended the anticipated radioactive yield and contaminated Japanese fishermen outside of the expected fallout zone—President Dwight D. Eisenhower and the Soviet Union leadership struggled with the terminology of agreements and details of a moratorium on nuclear testing. Scientists from both sides of the Iron Curtain finally met during the summer of 1958 to debate test ban issues and put recommendations in place for a temporary moratorium.

In fall 1958, at the height of the Cold War, the US Navy Bureau of Medicine and Surgery recommended establishing the Armed Forces Radiobiology Research Institute (AFRRI) at the National Naval Medical Center in Bethesda, Maryland, to research the biological effects of nuclear radiation. The AFRRI plans included construction of a nuclear reactor specifically designed to study the effects of ionizing radiation on humans. The AFRRI proposal was initiated, in large part, to alleviate the concern that if the moratorium on nuclear testing persisted, biomedical research and training on the physiological effects of irradiation might be deemed irrelevant. To prevent this possibility, Congress approved the proposal. Groundbreaking for the AFRRI began in November 1960.

By the 1960s, the United States was entrenched in the Vietnam War, and in the wake of the Cuban Missile Crisis, the specter of nuclear annihilation was pervasive on both sides of the Iron Curtain. In 1960, France detonated a nuclear device in the Sahara desert; a year later, the Soviet Union violated the signed moratorium by exploding the AN602 hydrogen bomb in the Novaya Zemlya archipelago in the Arctic Ocean.

Construction and staffing of the AFFRI was not completed until January 1962. Given the unsettling events of previous years, the civilian and the military medical communities felt an urgent need to broaden their collective understanding of the impact of radiation on troops and civilians. Thus, the AFFRI's initial mission statement emphasized conducting more comprehensive radiobiological scientific research essential to the medical support of US military services, national welfare, and global well-being.

Military veterinarians were involved in the AFRRI's radiobiology research from its inception, instituting macroscopic evaluation and eventually histopathological analysis of laboratory animals exposed to varying degrees of ionizing radiation. By 1963, the AFRRI radiation pathology department's roles were well delineated, specifying department responsibility for originating and conducting research projects in histopathology, cellular biology, and hematology. The radiation histopathology department was tasked with pathological analysis of biological tissue specimens, including laboratory animal necropsies and gross characterization of observed lesions, as well as the microscopic interpretation of all collected and key target tissues.

In 1968, the AFRRI added an experimental pathology department to its research hierarchy; personnel from this department were responsible for conceiving and executing radiobiological research on a variety of laboratory animal species, including nonhuman primates, dogs, cats, rodents, rabbits, pigs, and various exotic species. Using these animal models, the department evaluated the acute effects of radiation on sensitive individual cells and complete organ systems, with follow-on study of recovery and residual pathology associated with the initial insult.

The AFRRI veterinary pathologist now occupies a permanent position within the Department of Veterinary Science: division chief for comparative pathology. The current mission of this division chief is unique within the DoD and includes a broad spectrum of research in (*a*) medical countermeasure

development evaluating pharmacological treatment modalities that prophylactically prevent or treat various pathologies associated with ionizing radiation injury; (*b*) biological dosimetry clinically assessing various animal models to establish high-precision analytical methods for triage and medical management of radiation victims; (*c*) combined injury examining the development of medical treatments for irradiated personnel whose exposure has been compounded by traumatic wounds, burns, hemorrhage, blast injury, and/or infection; and (*d*) internal contamination and metal toxicity evaluating not only the short- and long-term radiological and toxicological effects of embedded military metals, but also the treatment strategies for improved elimination of said metals from the body.

Lessons Learned in Radiation Pathology

Many well-known and well-documented incidents involving human casualties have provided inadvertent data for better understanding of radiation pathology. Lessons have been learned through military use of nuclear weapons, as in the 1945 decision to drop a nuclear bomb on Hiroshima and Nagasaki, Japan, to bring an abrupt halt to World War II; from nuclear power plant disasters, as in Chernobyl of the former Soviet Union in 1986 and in the Windscale fire in Great Britain in 1957; from the intentional destruction of nuclear sites, as in the Iranian bombing of the Al Tuwaitha nuclear complex in Iraq during the Iran-Iraq war of the 1980s; and from accidental mishandling of nuclear material, as seen in the 1987 Goiania radioactive contamination accident in Brazil.[136–138]

The research afforded by these unfortunate incidents reveals that the pathophysiological effects of ionizing radiation are silent and initially painless, unlike most other injuries to the soldier. However, ionizing radiation manifests both acute and chronic effects, attacks a single or multiple body systems, and causes primary as well as bystander effects.

Division of Radiation Syndromes

Radiation pathology is divided into three overlapping, dose-dependent, clinical, and histopathological syndromes caused by any large, external penetrating dose of radiation delivered to the entire body (or most of it) over a short period of time. Acute radiation syndrome (ARS) is the collective term for the three syndromes caused by varying doses of radiation exposure.

The first ARS syndrome is hematopoietic syndrome (more commonly known as bone marrow syndrome). Mild hematopoietic syndrome symptoms such as nausea and vomiting have been described at doses as low as 0.3 Gy of ionizing radiation; more acute symptoms usually occur with single doses greater than 0.7 Gy. The higher the radiation dose, the more DNA damage is done to the bone marrow and to the blood cells (ie, red cells, white cells, and platelets) produced within the marrow. The survival rate of affected victims is also proportionally related to the amount of damage that occurs within the rapidly dividing bone marrow cells. As mature white blood cells start to turn over without having a regenerative pool of replacements, systemic thrombocytopenia (ie, decreased blood platelets and lowered clotting capabilities) and immunosuppression develop (Figures 15-6a and 15-6b). The primary cause of death is hemorrhage and infection.[139–145]

The second ARS syndrome, gastrointestinal syndrome or GI syndrome, usually occurs at higher single radiation exposure doses ranging from 10 to 100 Gy. Survival from this syndrome is rare because mucosal stem cells in the GI tract are destroyed. GI tract ulceration follows, enabling bacteria to invade the bloodstream. The most critical effects of this irreparable damage are sepsis and mucosal cell nonregeneration, which leads to GI absorption problems, dehydration, intractable diarrhea, severe electrolyte imbalances, and usually, death.[139–144]

The third ARS syndrome, termed as the cardiovascular or central nervous system syndrome, is typically observed at doses greater than 50 Gy and is the most fatal of the three syndromes. No recovery is ever expected; death occurs within 3 days secondary to total cardiovascular collapse associated with severe intracranial edema, disseminated necrotizing vasculitis, meningitis, and neuronal necrosis and loss.[139,140,142,143,144]

These syndromes are not static; the pathology frequently overlaps from one to the next. A significant amount of research has been dedicated to the study of all organ systems and tissues in the body, in isolation with targeted irradiation and in bodily functions as a whole, and in conjunction with blast, thermal, and other injury modalities such as heat, shock, and blast, often associated with nuclear device detonations. In addition, there is ongoing research in multiple organ systems examining the many late effects of insult with varying doses of ionizing radiation exposure.[136,138–140,144]

The veterinary pathologist is integral to several components of radiobiological research. Typically, studies culminate with histological evaluation of multiple body systems in the animal model, including an assessment of the impact of varied forms of insult and the viability of proposed countermeasure treatment.

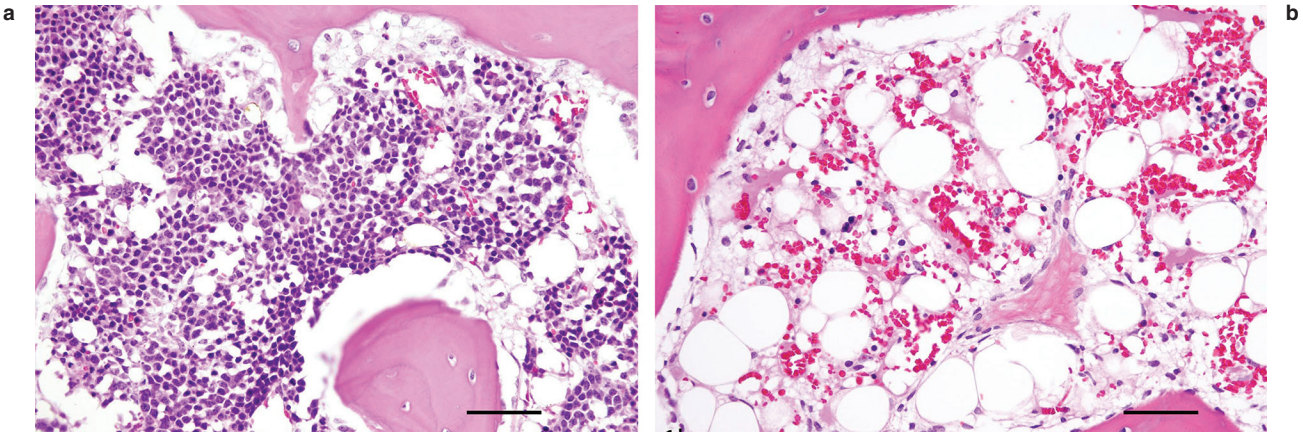

Figure 15-6: Bone marrow sections to show the effects of ionizing radiation. (a) Gottingen minipig, sternum, bone marrow: Normal population of myeloid and erythroid precursors. HE Bar = 50μm. (b) Gottingen minipig, sternum, bone marrow: Diffuse atrophy and loss of myeloid and erythroid bone marrow elements, with rare regenerative foci. HE Bar = 50μm. Photomicrographs courtesy of Lieutenant Colonel Eric D. Lombardini, chapter author.

Late Effects of Ionizing Radiation

Much has been published about the carcinogenetic effects of ionizing radiation on the body. Literature topics range from the primary effects of such exposure on atomic bomb survivors to how therapeutic exposure can also lead to disease in patients (ie, acquired secondary or bystander effects of exposure to clinical radiation). For example, ample evidence indicates victims of nuclear explosions face an increased risk of developing hematopoietic neoplasia (ie, leukemia) and a propensity for solid tumor formation that correlates to ionizing radiation exposure; this propensity affects almost all body systems.[138,140,142–144,146–148]

The literature also includes debate on how radiation affects cells. *Radiation carcinogenesis* occurs when a cell's genome is affected directly or indirectly by ionizing radiation. Various genetic mutations result, and, if maintained through multiple generations of cell turnover, can manifest as either tumor-promoting oncogenes or as defects in tumor suppressor genes that give rise to a monoclonal proliferation of the affected target cell and, ultimately, to neoplastic transformation.[138–140,148–152]

There are two primary opinions involving radiation-induced carcinogenesis. The more traditional of the two, "target theory," is based on the idea that all radiation-associated changes originate within the target cell. As a result, only those cells directly exposed to ionizing radiation maintain the necessary genomic alterations for oncogenesis.[139,140,153]

Alternatively, an evolving paradigm suggests that downstream, or bystander, effects occur at very low radiation doses and are associated with altered intercellular signaling pathways. This theory postulates that molecular changes do not just appear in irradiated target tissue; cells not directly injured by irradiation also can undergo multiple molecular modulations after receiving signals from cells originating within the field of injury. According to this paradigm, the cells that receive these signals from the irradiated target tissue exhibit multiple downstream effects, including mutative responses, genomic instability, gene induction, cell transformation, and cellular apoptosis.[139,140,153–156]

Downstream or bystander theory is important because it helps medical personnel to examine the effects of radiation on the organism as a whole and the evolving pathology as a continuum. This theory also blurs the lines between the specific categories proposed in the more traditional radiation syndrome model, allowing clinicians to treat the whole body, not just tissues of interest at certain stages of illness.

Late Effects of Depleted Uranium and Other Military Metals

Depleted uranium (DU) is an extremely high-density variant of Uranium-235 often derived as a by-product of uranium enrichment for nuclear energy or nuclear weaponry. DU can be used by civilian aircraft industries and for radiation shielding because of its unique physical properties. Its military applications include armor plating in vehicles (eg, tanks) and in armor-piercing munitions. Multiple friendly-fire incidents in which veterans sustained injuries with shrapnel-derived, embedded DU fragments were reported in August 1990 through February 1991 in

the Persian Gulf War. These included events in which US service members in armored vehicles were fired upon with DU penetrator, which produce variably sized shrapnel upon impact and aerosolized particulates.[157,158]

Little information is currently available about the potential long-term health consequences of such chronic, low-dose radiation exposure from these embedded fragments or on the combined effects of radiation and heavy metal toxicity. Ongoing animal studies continue to focus on the long-term effects of low-level radiation exposure on carcinogenesis and the ancillary effects on individuals in whom shrapnel is left in place, rather than surgically removed. Information from these studies is critically needed to determine appropriate medical management of DU fragment injuries and to decide if these sustained injuries require an alternative treatment, different from treatments for other embedded metal fragments.

Studies have examined the effects of other metals used in military munitions, however. In one notable group of experiments, AFRRI scientists observed how tissues in rats' leg muscles were affected by embedded tungsten fragments and discovered a significant late effect: a highly malignant rhabdomyosarcoma developed around the embedded tungsten fragment[159] (Figure 15-7).

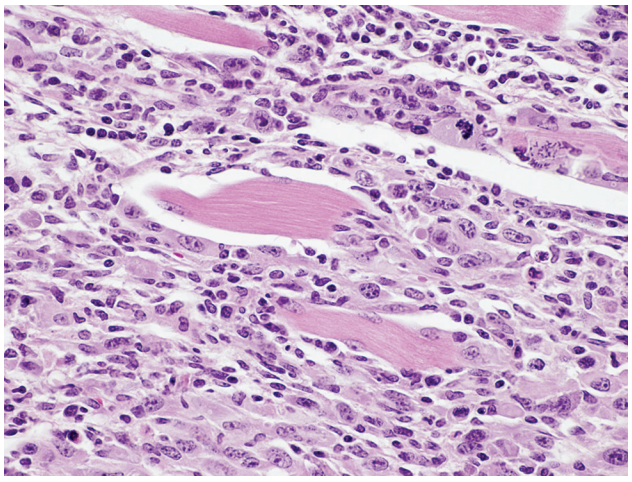

Figure 15-7: Rat, leg: Secondary to the experimental implantation of a military metal (tungsten) pellet into the leg of this animal, a rhabdomyosarcoma developed. The photomicrograph displays the few surviving normal skeletal muscle myocytes that are separated, surrounded, and replaced by neoplastic cells. HE 400x.
Photomicrograph courtesy of Lieutenant Colonel Eric D. Lombardini, chapter author.

Complications from Combined Injuries

A combined injury is defined as physical, thermal, and/or chemical trauma combined with radiation exposure at a dose that diminishes recovery and survival chances. In other words, a combined injury patient's prognosis is more critical than those patients diagnosed with trauma alone or radiation exposure alone. To illustrate this heightened effect, consider a terrorist attack using a dirty bomb (ie, a combination of conventional explosives and radioactive material) within a subway system or another urban setting. The immediate impact of the actual radiation exposure would be obscured by the primary, instantaneous blast overpressure effects of the explosive detonation and the associated injuries sustained by airborne shrapnel and debris. A victim exposed to ionizing radiation probably would be subject not only to consequential thermal and/or radiation burns and internal organ hyperthermia, but also to blunt force and blast wave trauma and bodily lacerations that could obscure the initial clinical and histopathological picture of the radiation exposure effects.

Use of Medical Countermeasures

After a military tactical strike or natural or man-made disaster, first responders and occasionally military troops are required to enter into contaminated areas and function safely within that zone for an unpredictable amount of time. A clear understanding of the pathological effects associated with the radiation exposure syndromes, as well as an appreciation of potential long-term effects of any exposure, are critical in mission design and deployment, victim triage, and casualty treatment. Over the years, AFRRI personnel have examined the viability of hundreds of potential medical countermeasures that could be used (*a*) as prophylactic treatment for the military or first responders who might be required to enter into radiological- or nuclear-contaminated areas or (*b*) as postexposure treatment for individuals who have already received high doses of therapeutic or accidental ionizing radiation.

While the vast majority of research into finding radioprotective compounds has had limited success, the ongoing research at AFRRI has produced several investigational new drugs that are in various stages of development. Examples include granulocyte colony stimulating factor and different tocotrineols members of the vitamin E family, which have been evaluated *in vivo* in rodents and to a lesser degree in nonhuman primates. The military veterinary pathologists assigned to the institute also have been instrumental in

examining the histopathological changes associated with varying degrees of irradiation combined with a range of countermeasure doses. This examination sheds light not just on efficacy, but also on potential toxicology and optimal dosing in humans and various other animal species.[160–163]

Future Demand for Radiation Studies

World-wide tensions about nuclear events have heightened since the March 2011 combined natural and man-made disasters in Japan. (A powerful earthquake off the Pacific Coast of Tohoku led to violent tsunami flooding, which in turn, caused a nuclear meltdown and release of radioactive materials at the Fukushima Daiichi nuclear power plant.) These anxieties are exacerbated by other international circumstances, including the persistent nuclear threats posed by an unstable North Korean government, the nuclear aspirations of an absolute Iranian political regime, and a laxity of control over Pakistani and former Soviet republic nuclear stockpiles.

Closer to home, the threat of a terrorist nuclear incident also remains an ongoing concern for the DoD. In the absence of readily available and effective commercial radiation medical countermeasures and minimally invasive biodosimetric tools, the AFRRI must continue its primary mission of using radiobiology research to develop beneficial medical products for soldiers, and military veterinary pathologists need to remain collaborating partners with civilian research scientists to extend similar protection to US citizens.

COMBAT CASUALTY CARE

Another important branch of ongoing veterinary military pathology research involves the physiological impact of traumatic injury, burns, and blast trauma. The US Army Institute of Surgical Research (ISR), located in San Antonio, Texas, is the DoD's lead agent for combat casualty care, focusing on hemorrhage, tissue injury and trauma, resuscitation, medical devices, and clinical research. With the mission of providing requirements-driven medical solutions and products for injured soldiers—from self-aid through definitive care across the full spectrum of military operations—the ISR is also the DoD's only full-service animal research facility that is co-located with a Level 1 trauma center. Thus, the ISR allows for professional scientific collaboration between veterinary pathologists, human pathologists, trauma surgeons, pain medicine providers, dental surgeons, and combat casualty care researchers.

Evolving Missions of the United States Army Institute of Surgical Research

The ISR, originally named the surgical research unit, was established in 1943 to evaluate the role of antibiotics, which had just been discovered as war wound treatments. The unit was first stationed at Halloran General Hospital, Staten Island, New York. In 1947, the IRS became a permanent unit and moved to Brooke General Hospital, Brooke Army Medical Center, Ft Sam Houston, Texas. It was assigned 12 personnel to continue research on antibiotics and to begin studying innovative surgical techniques and developments.

In 1949, given the potential for numerous nuclear weapons casualties, the unit's mission expanded to include evaluation of thermal injuries. Study of these injuries led to improved skin grafting procedures and promoted continued use of antibiotics in new applications. During the 1950s, the ISR also served as a premier dialysis research center in South Central Texas and neighboring states.

The ISR was assigned to headquarters, US Army Medical Research and Development Command, in September 1958. Although it is colloquially known as the "Army's Burn Unit," the IRS serves all service branches and is a prototype for burn units all over the world. The ISR is also responsible for many forward-thinking medical research initiatives, including using plasma extenders and grafting and preservation of blood vessels.

As part of an AMEDD reorganization in March 1994, the ISR became a subordinate command of the MRMC, which is a major subordinate command of the newly formed US Army Medical Command. In 1996, the ISR moved to its current location, adjacent to the newly constructed Brooke Army Medical Center (now named the San Antonio Military Medical Center). Its current mission focus has changed from burn care management and treatment of thermal injuries to equal emphasis on the full spectrum of combat casualty care, including providing medical solutions for the injured soldier on the battlefield. Ongoing construction of the Battlefield Health and Trauma Research Institute, authorized by the 2005 base realignment and closure directive, will help consolidate all DoD combat casualty care research and personnel with the ISR at the San Antonio Military Medical Center.

Veterinary Pathology Contributions to Combat Casualty Care

Veterinarians have been involved at each step of the ISR's changing mission: from cutaneous burn treatment, systemic burn therapy modalities, and pulmonary

burn injuries to broader battlefield trauma. In fact, VC officers contributed to burn research since its inception in the late 1940s, and the first board-certified veterinary pathologists began studying wound infection in the late 1970s.[164]

When the ISR's mission expanded to include inhalation burn injuries, Dr Gene Hubbard introduced sheep as the model of inhalation injury and published over 15 manuscripts presenting research results and treatment implications for soldiers recovering from smoke inhalation. His studies suggest that if initial toxic injury and inflammation can be controlled to prevent pneumonia, mortality rates should be reduced. Retired Army Colonel Basil A. Pruitt, Jr, MD, FACS, commander and director of the ISR for 27 years, summarized the overall contributions of veterinary pathologists such as Hubbard as follows: "they were true participants and added value in the research" and "they were key in the developments in wound infection, inhalation injury, and skin and tendon grafts" (oral communication, San Antonio, Texas, 2013**).**

More recent contributions by veterinary pathologists include research on Factor VII, a key component of the extrinsic clotting cascade[165]; topical hemostatic agents[166]; blood replacements[167]; tourniquets[168]; and safety evaluation of new hemostatic agents such as clay mineral smectite granules and kaolin-coated gauze in a vascular injury wound model in swine.[169–171] Over 30 patents have been granted, and five designations for "Army Invention of the Year" have been awarded as a result of recent ISR medical countermeasures. These countermeasures also prompted life-saving modifications to the first-aid kits carried by deployed combat soldiers. Because of these changes, soldiers can now render self- and buddy-aid stabilization until definitive care is provided following evacuation to DoD and civilian hospitals.

FIELD OPERATIONS

Early Missions of Veterinary Pathologists

Much of the support mission of military veterinary pathologists is provided behind microscopes within the confines of various research institutes. Still, throughout history, military veterinary pathologists have served as field diagnosticians in more remote locations worldwide and near the front lines of global combat missions. Although the majority of this service is performed in the background (ie, assisting commanders to detect zoonotic diseases early—before troops are affected), deployed soldier-scientists have also undertaken more prominent roles to safeguard the combat strength of US and allied forces.

Several missions that veterinary pathologists have spearheaded warrant mentioning. One notable mission began decades ago when the AFIP developed an exchange program with the Onderstepoort Veterinary Institute (OVI) in South Africa. This cooperative venture lasted from 1963 to 1987; during its tenure, many US veterinary pathologists worked, conducted research, and lived in South Africa.

The OVI was established in 1908 by Swiss veterinarian Sir Arnold Theiler in the wake of a smallpox epidemic among miners in the Witwatersrand region of South Africa. The institute was founded as a center for diagnostics and vaccine production, and, under Theiler's direction, it conducted research and prevention work on rinderpest (ie, morbillivirus), African horse sickness (ie, orbivirus), sleeping sickness (ie, African trypanosomiasis), malaria, East Coast fever (ie, *Theileria parva*), and various tick-borne diseases, including babesiosis and heartwater (ie, *Ehrlichia ruminantium*).[172]

The first military participant, Dr Robert M. McCulley, was assigned to Onderstepoort between 1963 and 1969. During his tour of duty, he studied parasitic infections of hippopotami in the Kruger National Park and took part in the discovery of *Besnoitia* cysts in blue wildebeest, leading to the production of a live vaccine to protect cattle against the disease.[173] He also studied hepatozoonosis in carnivores,[174] cytauxzoonosis in giraffe,[175] herpesvirus in elephants,[176,177] and the parasites of kudu, bushbuck, and African buffalo.[178,179]

During their tours of duty at the OVI, subsequent military veterinary pathologists Dr Gene McConnell, Dr George Imes, and Dr John Pletcher collectively studied, catalogued, photographed, and published on the myriad diseases to be found in the South African wildlife. Examples of their work included the extensive study of baboon diseases, driven by an outbreak of Marburg virus in African green monkeys in Germany. The DoD was concerned about the potential introduction of a similar disease into the United States through wild-caught baboons destined for use in flight research.

While antibodies to the Marburg virus were not found in the native baboons, Dr McConnell's extensive work on the Chacma baboon allowed the description of a novel parasite-host interaction upon the identification of coccidial oocysts within the skeletal muscle.[180,181] Similarly, McConnell published manuscripts on nasal and laryngeal acariasis[182,183]; on cardiac, cerebral, and skeletal toxoplasmosis in baboons[184]; and on a case of reverse zoonoses in which myocardial tuberculosis was diagnosed in a baboon[185] that was exposed during capture.

Research by US military veterinary pathologists in South Africa was not limited to nonhuman primates, however. McConnell also reported about a case of anthrax in the African buffalo[186]; studied the pathology of exertional rhabdomyolysis in humans and both domestic and wild-caught animals[187]; and collaborated in the diagnosis and description of an abortion epizootic due to vibriosis in sheep.[188] Additionally, his work was pivotal to the identification and control of a transboundary foot and mouth disease outbreak in a region where eradication was deemed impossible due to the local populations' cultural mores (oral communication, Washington, DC, May 22, 2012).

Later, images and materials garnered from McConnell's OVI field operations were contributed to the AFIP and to the foreign animal disease diagnosticians course offered on Plum Island, New York. At this course, veterinarians from multiple areas of specialization and expertise are given hands-on training and familiarization with diseases not currently present in the United States, but which have high likelihood to be disastrous to the US agriculture economy should they arise in the United States.

While stationed at the OVI, Dr Imes conducted innumerable necropsies on hyena and lions; published manuscripts on initial descriptions of bovine protothecosis[189] and vitamin A deficiency in a lion cub[190]; examined and evaluated a trout mortality event due to streptococcus[191]; and reported the presence of coccidiae in the viscera of Nile crocodiles.[192] In one case, he reported on an interesting and obscure parasite behavior in which ticks congregated on lions' ventral midlines in what was termed "tick islands." He subsequently correlated the presence of ticks as the vector for blood-borne microfilaria that, on further study, were found microscopically present within sectioned ticks (oral communication, Washington, DC, May 22, 2012).

Dr Pletcher researched South African impala and warthogs, performing necropsies of these animals as part of a parasitology and disease survey of both species. The vast amount of data collected enabled the description of two associations: (1) between the nematode *Cooperioides hepaticae* and hepatic disease in impala[193] and (2) between the nodular abomasitis in impala lambs and the nematode *Longistrongylus sabie*.[194] Because of this work, a new species of filarial worm found microscopically infecting the lymph nodes of warthogs was also described.[195]

Emerging Force Protection Efforts

Although OVI research added much to the collective body of knowledge about animal disease, OVI data contributed more to homeland agricultural and economic defense. OVI study results continue to guide US preparations against emerging foreign animal diseases. By extrapolating from these earlier South African studies and through the study of both the macroscopic lesions photographed by these pathologists and the extensive archive of histopathological slides that they generated, the US Department of Homeland Security and the USDA have trained hundreds of civilian and military veterinarians to recognize and diagnose animal diseases foreign to US soil.[196]

Other veterinary pathology research has focused more specifically on force protection. For example, Dr Thomas Bucci, a veterinary pathologist who conducted missions in Egypt and the Sudan, concentrated on managing and controlling an epidemic of Rift Valley fever, a virus that affects humans and livestock. While Bucci's primary responsibility of tracking arthropod-borne viruses was not the standard mission directive for a veterinary pathologist, he collected blood samples from animals and humans and identified 21 separate arthropod-borne viruses, including WNV, yellow fever, and Rift Valley fever viruses. He also conducted necropsies on, and scrutinized hepatic biopsies of, camels managed by the Egyptian Camel Corps. His research led to a more complete understanding of the epidemiology of a series of viral diseases of military importance and recognition of the zoonotic risk to deployed troops within the region (oral communication, Washington, DC, May 14, 2012).

Another mission with tangible connections to the battlefield is that of the Army veterinary pathologist assigned to the 520th Theater Area Medical Laboratory (TAML), activated in October 1995. The TAML was designed to be highly flexible and thus deployable: it was uniquely modular, tailored to varied missions, and equipped with high-tech, cutting-edge capabilities. In this unit, a military veterinary pathologist was embedded with a team of other military scientists because of the dual skill set the veterinary pathologist possessed: the veterinarian's familiarity with diseases afflicting animal and human populations and the pathologist's specialized ability to detect, identify, and describe diseases.

The TAML's initial assignment was in December 1995 when it deployed to support stabilization forces in Bosnia-Herzegovina. It deployed again in the spring of 1998 to Kuwait as part of Operation Southern Watch. Army veterinary pathologists, in collaboration with biochemists, microbiologists, entomologists, and other AMEDD scientists, were sent on both missions to provide early gross and histopathological detection; diagnosis and confirmation of zoonotic diseases if biological warfare agents were employed in the area of operations against allied military forces; and assessment and diagnosis of environmental health risks.

At the onset of Operation Iraqi Freedom in March 2003, the TAML was embedded in the 86th Combat Support Hospital convoy heading north from Kuwait into southern Iraq. The convoy took approximately 2 days to reach the Iraqi Tallil Airbase, halting frequently to take protective posture from flyovers by tactical ballistic missiles (ie, SCUDs). For 10 months until redeployment in December 2003, the Army veterinary pathologist assigned to the TAML was involved in the unit's fluid combat mission (Jo Lynne Raymond, Colonel [Retired], oral communication, Washington, DC, May 10, 2012).

The initial mission was to identify any potential weapons of mass destruction and to use rapid diagnostic assays to protect coalition forces if CBRN weapons were employed. When TAML personnel did not uncover any weapons of mass destruction, they addressed existing threats within the combat environment that might be significantly detrimental to the deployed troops' combat efficacy, such as leishmaniasis.

Leishmaniasis is a protozoal disease responsible for significant morbidity among US military forces and allies throughout history. The organism is transmitted by the bite of phlebotomine sand flies, a species that also transmits the etiological agents of bartonellosis and pappataci fever. During World War II, about 1,200 cases of cutaneous leishmaniasis and 75 cases of visceral leishmaniasis were reported among Allied troops stationed in the Middle East. Both Israeli forces operating in the Jordan Valley during the 1967 Arab-Israeli Six-Day War and present-day personnel determined to be at risk among the multinational force and observers stationed in the Sinai Desert have experienced very high rates of leishmaniasis (50% among the former and 20% among the latter).[197]

From March 2003 to November 2004, the initial days of Operation Iraqi Freedom, 1,178 cases of cutaneous leishmaniasis were diagnosed in US military personnel, not accounting for civilian contractors, allied troops, or inherent underreporting by personnel afflicted with the disease.[198] Cutaneous leishmaniasis is also endemic in Afghanistan. The World Health Organization reported a significant resurgence of the disease since the early days of Operation Enduring Freedom in 2002. At that time, at least 250,000 individual cases were reported nationwide in Afghanistan, and of those, at least 200,000 were reported in Kabul alone.[197] (Cutaneous leishmaniasis typically presents as a self-limiting, ulcerative, and nodular dermatitis that can resolve into severely disfiguring scars.)

Early epidemiological medical assessments conducted prior to sending troops into Iraq did not initially account for the actual incidence rate of leishmaniasis encountered in theater. These assessments also failed to predict that sand flies would find a perfect habitat within the tent cities erected by the US military early in the conflict. Because of these mistakes, high numbers of military troops deployed without the necessary personal protective and preventive equipment; therefore, the exposure risk was extremely high. Even when troops did arrive with the appropriate gear, the extremely harsh environment and stifling heat resulted in personnel not using their protective equipment properly.

Scientists assigned to the TAML attacked the leishmaniasis problem cooperatively, capitalizing on the various medical specialties present and subdividing the tasks of identifying, understanding, controlling, and preventing the disease. For example, the TAML veterinary pathologist assisted preventive medicine entomology personnel, conducting an extensive assessment of the vectors and reservoirs of the disease.

All of the local wildlife, including feral dogs that roamed the base in packs, were assessed for the presence of leishmania. Necropsies were performed on trapped feral dogs, rodents, and small insectivores such as hedgehogs, in addition to a few lizards and snakes. Information garnered from histopathological assessment of tissues collected and local disease surveys established a map of animal reservoirs that eventually enabled construction of a risk assessment profile.

Such epidemiological studies help the DoD, especially in longstanding conflicts in which large numbers of troops move in and out of the theater of operations, potentially ending up in the military medical system once redeployed back to their home station. These epidemiological assessments educate military hospitals and the civilian medical system to ensure appropriate and quicker treatment of veterans who present with clinical signs of a foreign country's endemic disease.[199]

In addition to the leishmania-specific postmortem surveys, military veterinarians conducted multiple humanitarian missions among the local Shi'ite tribesmen over the course of several months. While other military personnel conducted clinics for the Iraqi populace, veterinary officers performed herd health medicine by deworming sheep flocks; conducted basic field medicine on camels and a few cattle, chickens, and goats; and occasionally treated the rare semidomesticated dog or cat. When appropriate, veterinary officers also performed euthanasia and necropsy of severely ill animals and collected samples for histopathological assessment to add to the database on disease prevalence. The data collected on domestic animal disease prevalence is now proving invaluable; nongovernmental agencies rebuilding Iraq are using the accumulated information to improve the effectiveness and efficiency of their assistance.

The 502nd TAML elements were inactivated following deployment to Iraq in 2003 and reorganized into the more modular 1st and 9th Area Medical laboratories. These smaller deployable units are structured with the specific mission to provide rapid on-site diagnostic capabilities for the purpose of early health threat detection and confirmation and medical surveillance of various CBRN threats, as well as conditions associated with occupational, environmental health, and endemic diseases (Jo Lynne Raymond, Colonel [Retired], oral communication, Washington, DC, May 10, 2012).

SUMMARY

As this chapter briefly illustrates, the role of military veterinary pathologists is, by its very nature, dynamic. Military veterinary pathologists represent a small but diverse group of soldier-scientists whose research is necessary in an increasingly unstable world.

Since the end of the Cold War, ongoing diffusion of power from the bipolar geopolitics of the 1980s, which was governed by the United States and the Soviet Union, has developed into a global tapestry of failed nation states, such as the former Yugoslavia, Somalia, and Zimbabwe; extremist regimes within nations, such as Sudan, Niger, and North Korea; and political posturing by nations, such as Iran and Venezuela. Losing control of CBRN weapon stockpiles and regional instability also remain of critical concern in regions of India and Pakistan.

The world is also subjected to a revolution of technology and information, with terrorists possessing quick and easy access to both. Because of such instantaneous global threats, the US military is likely to continue devoting significant resources, strategic emphasis, and dedicated manpower to develop a protective shield against future attacks. In their mission to enable, support, and help evolve military research into effective medical countermeasures to combat modern CBRN threats, veterinary pathologists rely heavily on the research and the trained personnel provided by the DODVPR and on diagnostics. Military veterinary pathologists also seek to develop a better understanding of disease pathogenesis by studying the impact of these dangers in various government-owned laboratories and in combat and field environments.

Finally, the military veterinary pathologist has been a defender of the soldier and citizen in the past and will continue this role in the future. Pathologists are integral to basic research and discovery of new pathogens, vital to drug development, and critical to developing pertinent animal models for vaccine and therapeutic safety and efficacy. Contributions to scientific literature made by veterinary pathologists include reference texts, peer-reviewed journal manuscripts, military field manuals, and technical bulletins. These publications disseminate scientific discovery and crucial preventive and interventional strategies to promote biodefense and biomedical research.

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